Mechanisms of genetic differentiation among seabird populations

Anicee Jessica M. Lombal

Discipline of Biological Sciences

School of Natural Sciences

University of Tasmania

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Thesis abstract

Connectivity among populations is considered beneficial for population persistence, reducing levels of inbreeding, increasing genetic diversity, and potentially providing demographic and fitness benefits. Consequently, predicting gene flow between populations based on factors such as wind-dispersed seeds in plants, or presence of pelagic larvae in fishes, is highly desirable for identifying conservation priorities and maintaining viability of species. Seabirds provide useful model systems for studying the factors influencing gene flow given their discrete breeding distributions. As little is known about the non-physical (biotic) factors affecting movement among oceanic seabird colonies, my research addresses this crucial knowledge gap in the context of maximizing persistence and resilience of seabird species. This new knowledge can be extended to other organisms in further studies.

The aims of my Ph.D. project are to provide practical case studies to investigate contemporary mechanisms of genetic differentiation among seabird colonies. To explore how these and other factors explain seabird population genetic differentiation, I combined my results with those from over 71 studies reporting population genetic data for seabird species and performed a meta-analysis.

In my first case study, I tested for a relationship between differences in non-breeding distributions and genetic structuring of flesh-footed shearwaters A*rdenna carneipes*, a migratory species nesting at Lord Howe Island, New Zealand, southwestern Australia and Saint-Paul Island in the Indian Ocean. Telemetry studies suggest that eastern and western colonies migrate to different non-breeding grounds (North Pacific Ocean and northern Indian Ocean, respectively), and segregation based on migratory patterns has been hypothesised to

contribute to population genetic divergence. My results showed strong genetic differentiation between Pacific colonies relative to those to the west. However, molecular analyses of fisheries' bycatch individuals sampled in the North Pacific Ocean indicated that individuals from both eastern and western colonies were migrating through this area. As the apparent segregation of the non-breeding distribution based on telemetry was not corroborated by my genetic analyses, I concluded that this factor was not a contributor to the population genetic structure observed among colonies.

In a case study of a second species, I tested whether genetic isolation exists among colonies that exhibit other phenological or circadian differences—e.g. diurnal versus nocturnal colony attendance. The providence petrel *Pterodroma solandri*, is an oceanic seabird restricted to two breeding colonies off eastern Australia: Lord Howe Island, and a recently discovered colony on Phillip Island (adjacent to Norfolk Island). Historically, the providence petrel also nested on Norfolk Island, comprising ~ 1 million breeding pairs, before becoming locally extinct by the late 18th century. The reasons for extinction include exploitation by European settlers subsequent to 1788 and the introduction of mammalian predators. The two extant colonies show different times of return to nesting sites (diurnal versus nocturnal), which may represent local adaptation that could inhibit dispersal between populations. I used genetic data to investigate connectivity between these colonies. My results showed genetic homogeneity of colonies, indicating that the small population on Phillip Island represents a recent colonization from the Lord Howe population rather than a relic population from the geographically closer but now extinct Norfolk population. Hence, it is likely that prospectors from Lord Howe Island or their descendants have switched their behaviour on Phillip Island.

In a separate study, I analysed subfossils of providence petrel from the extinct (Norfolk Island) population to assess whether population extinction occurred in the presence of genetic connectivity, which is essential to assess the limits of connectivity to attenuate processes that have driven extinctions. The majority of subfossil Norfolk Island individuals exhibited the most common mitochondrial haplotype from Lord Howe Island, consistent with high genetic connectivity. This study provides an insight into how rapidly even very large seabird populations can be decimated by humans despite genetic connectivity with unperturbed populations, which has significant conservation implications for predicting the resilience of other species.

I then incorporated my case studies into a multi-species dataset of genetic variation among seabird colonies. I evaluated a candidate set of generalized linear models (GLMs) to identify contributors to population genetic differentiation for these 73 seabird species. Historical fragmentation was the best predictor of genetic differentiation within seabird species and was supported by variation in phenotypic traits, whereas non-physical barriers such as differences in non-breeding movement patterns among colonies did not appear a significant predictor of genetic structure. These results show that signatures of historical events still dominate as contributors to contemporary genetic structuring among seabird colonies even if they are not enduring, provided that they are subsequently reinforced by factors such as constraints on foraging imposed by spatial heterogeneity of ocean productivity.

Statements by the author

Thesis declaration

I certify that this thesis contains no material which has been accepted for a degree or diploma by the University or any other institution, except by way of background information and duly acknowledged in the thesis, and to the best of my knowledge and belief no material previously published or written by another person except where due acknowledgement is made in the text of the thesis, nor does the thesis contain any material that infringes copyright.

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Statement of Ethical conduct

The research associated with this thesis abides by the international and Australian codes on human and animal experimentation, the guidelines by the Australian Government's Office of the Gene Technology Regulator and the rulings of the Safety, Ethics and Institutional Biosafety Committees of the University.

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Anicee Jessica M. Lombal Date

Statement of co-authorship

The following people contributed to the publication of work undertaken as part of this thesis:

Anicee Lombal (Candidate), Discipline of Biological Sciences, University of Tasmania, Hobart, Australia

Christopher Burridge, *Supervisor 1*, (Author 2), Discipline of Biological Sciences, University of Tasmania, Hobart, Australia

Eric Woehler, *Supervisor 2,* (Author 3), Institute of Marine & Antarctic Studies, University of Tasmania, Hobart, Australia

Jeremy Austin, (Author 4), Australian Centre for Ancient DNA, School of Biological Sciences, University of Adelaide, Adelaide, Australia

Theodore Wenner (Author 5), Discipline of Biological Sciences, University of Tasmania, Hobart, Australia

Jennifer Lavers (Author 6), Institute of Marine & Antarctic Studies, University of Tasmania, Hobart, Australia

Nicholas Carlile (Author 7), Department of Environment and Conservation, Hurtsville, Australia

David Priddel (Author 8), Department of Environment and Conservation, Hurtsville, Australia

Ian Hutton (Author 9), Lord Howe Island Museum, Lord Howe Island, Australia

Kieren Mitchell (Author 10), Australian Centre for Ancient DNA, School of Biological Sciences, University of Adelaide, Adelaide, Australia

Alexander Salis (Author 11), Australian Centre for Ancient DNA, School of Biological Sciences, University of Adelaide, Adelaide, Australia

Alan Tennyson (Author 12), [3]Museum of New Zealand Te Papa Tongarewa, Te Aro, New Zealand

Lara Sheperd (Author 13), [3]Museum of New Zealand Te Papa Tongarewa, Te Aro, New Zealand

Trevor Worthy (Author 14), Biological Sciences, Flinders University, Adelaide, Australia

Vicki Friesen (Author 15), Department of Biology, Queen's University, Kingston, Canada

James O'Dwyer (Author 16), Discipline of Biological Sciences, University of Tasmania, Hobart, Australia

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The Candidate was the primary Author. The Candidate performed the sample collection with Author 6 and 9. The Candidate collected the molecular data with Author 5. The Candidate performed the genetic and statistical analyses. Author 2 and Author 6 designed the study. Authors 2–6 and 9 contributed to the manuscript.

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The Candidate was the primary Author. Authors 2, 4, 5, 7 and 8 sampled individuals. The Candidate collected the molecular data with Author 5. The Candidate performed the genetic and statistical analyses. The Authors 2, 3 and 7 designed the study and contributed to the manuscript.

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The Candidate was the primary Author. The Candidate collected the data with Author 16. The Candidate performed the genetic and statistical analyses. The Candidate and Author 2 designed the study. The Authors 2, 3 and 15 contributed to the manuscript.

We the undersigned agree with the above stated proportion of work undertaken for each of the above published (or submitted) peer-reviewed manuscripts contributing to this thesis.

Christopher Burridge

Primary Supervisor

Discipline of Biological Sciences

University of Tasmania

Signed

Date 21.11.2019

Mark A. Hunt

Head of School

Discipline of Biological Sciences

Signed

Date 2.11.2019

University of Tasmania

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I dedicate this work to my father, Michel Lombal, who passed away ten years ago as I like to believe that he would have been proud of this achievement.

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1.1 Background

Impact of genetic connectivity to the viability of species

Connectivity among populations is considered beneficial for population persistence, reducing levels of inbreeding, increasing genetic diversity, and providing demographic and fitness benefits (Frankham, 1996). For instance, Allendorf (1991) examined the effects of immigration on the rate of loss of genetic variation in an isolated population of grizzly bears in the Rocky Mountains and found that the introduction of a few bears per generation significantly reduced the rate of loss of variation in the isolated population. Another example is the empirical study by Spielman (1992) showing significant increases in reproductive fitness in ten replicate inbred lines of *Drosophila melanogaster* that received only one migrant per generation. Subsequently, since inbreeding reduces reproduction and survival (Frankham, 2005), low genetic diversity is expected to increase population extinction risk, especially by compromising evolutionary response to environmental changes (Frankel, 1974). Indeed, in a comprehensive meta-analysis, involving 170 paired comparisons, Spielman *et al*., (2004) found that the majority of threatened taxa (77%) exhibited reduced genetic diversity, which indicate that most threatened taxa are suffering reduced ability to evolve and consequent reduced reproductive fitness.

Migration of individuals, or gene flow, and the subsequent transfer of genes among populations can help prevent subpopulation isolation, thereby maintaining genetic variation and preventing inbreeding depression (Frankel and Soulé, 1981). On the other hand, the decision to augment gene flow in fragmented populations (e.g. management actions) is sometimes limited due to concerns about outbreeding depression (OD) (Edmands, 2007),

although the probability of OD in crosses between populations is much less than the probability of population extirpation dur to inbreeding depression and loss of genetic diversity in separate, small populations (Frankham *et al*., 2011). As a result, predicting gene flow among populations based on factors such as wind-dispersed seeds in plants (Hamrick *et al*., 1992), or presence of pelagic larvae versus direct development in fishes (Kyle and Boulding, 2000; Dawson *et al*., 2014), is essential for identifying conservation priorities and maintaining viability of species through assisted or restored dispersal (Greenwood *et al*., 1978; DeSalle and Amato, 2004; Proft *et al*., 2018). Yet, the determinants inhibiting genetic connectivity among populations, potentially leading to genetic divergence among them, remain unclear and more studies are required to investigate this topic.

Seabirds as model systems to investigate the mechanisms of genetic differentiation among populations

Seabirds are known for their ability to travel long distances, with members of some species travelling thousands of kilometres on a single foraging trip (Croxall *et al*., 2005). However, a number of studies showed evidence of strong genetic differentiation among seabird colonies (Burg and Croxall, 2001, 2004; Birt *et al*., 2011). This apparent contradiction between vagility and reluctance to disperse (change location of breeding) in seabirds, a phenomena often referred as the 'seabird paradox' (Milot *et al*., 2008), has raised a number of questions on the evolution of dispersal in the marine environment where foraging areas are often far from breeding sites. Indeed, while physical factors inhibit gene flow among seabird colonies (e.g. presence of land, Friesen *et al*., 2007), they do not provide a complete explanation of population differentiation in seabirds as genetic structure can also exist in the apparent absence of physical barriers (Friesen *et al*., 2007; Friesen, 2015). This suggests that contemporary processes, such as philopatric behaviour (Coulson, 2016) and adaptation to

different environmental conditions due to discrete breeding distributions – e.g. segregation in non-breeding distributions among colonies, Burg and Croxall, 2001 – are also important processes in population differentiation. Therefore, seabirds are excellent model systems to investigate the benefits of philopatry and coloniality (Coulson, 2002), the barriers to dispersal in the marine environment (Friesen *et al*., 2007), and the dynamics of colony formation and extinction (Matthiopoulos *et al*., 2005).

Potential determinants of genetic differentiation in seabirds

Among the non-physical (biotic) factors that have been suggested to inhibit gene flow among seabird colonies, philopatry has been often advocated (Friesen, 2015; Warham, 1990). However, a number of seabird species showing high rates of return to their natal breeding sites do not present genetic structure among populations (e.g. Ball and Avise, 1992; Pearce *et al*., 2004; Roeder *et al*., 2001). Conversely, inferences of high philopatry based on long-term banding records and recapture rates have been contradicted by genetic studies suggesting high rates of gene flow among colonies (e.g. Austin *et al*., 1994; Birt-Friesen *et al*., 1992; Ovenden *et al*., 1991). Therefore, philopatry is not always a predictor of population genetic structure or has not been accurately quantified in some species. Indeed, in a review of philopatry in seabirds in comparison with other waterbird species, Coulson (2016) showed that the rates of return to breeding sites are probably more variable than previously suggested as they are influenced by environmental conditions and population pressures, and so should not be considered a constant for individual species. Hence, many banding studies may have overestimated natal philopatry, which makes meaningful tests of relationship between philopatry and genetic structure difficult.

Another biotic factor that has been suggested as a predictor of gene flow among colonies is segregation in non-breeding distributions (e.g. Burg *et al*., 2003; Clucas *et al*., 2014; Gangloff *et al*., 2013). Migratory seabirds from different colonies often display discrete foraging distributions during the non-breeding season that may limit gene flow among populations and promote local differentiation. (e.g. Catard *et al*., 2000; Peck and Congdon, 2005). Therefore, seabirds that migrate to population-specific non-breeding grounds or are year-resident at colonies may have less opportunity for gene flow than those that have a shared or overlapping non-breeding distribution (e.g. Burg and Croxall, 2001; Gangloff *et al*., 2013). For example, Burg (2001) found that Black-browed albatrosses *Thalassarche melanophris* showing distinct foraging grounds during the non-breeding season differ genetically despite a lack of physical barriers to dispersal among colonies. However, segregation in non-breeding distributions among colonies *per se* is unlikely to always explain restrictions in gene flow among colonies as Friesen (2015) showed that 63% of species whose populations overlap in non-breeding distribution show evidence of restrictions in gene flow among colonies. Moreover, other studies found no relationship between foraging segregation during the non-breeding season between colonies and genetic structure (e.g. Quillfeldt *et al*., 2017). Hence, a greater number of studies are required to test whether differences in non-breeding distributions influence genetic divergence among seabird colonies.

Numerous studies have illustrated the importance of behavioural variation as a fundamental characteristic of seabirds living in spatially variable environments (e.g. Falk *et al*., 2002; Paiva *et al*., 2009; Reed *et al*., 2009). Variation in behaviour among colonies may inhibit gene flow among populations. One example is the Hawaiian petrel *Pterodroma sandwichensis* nesting on Hawaii and Kauai and foraging in different areas during the

breeding season (Wiley *et al*., 2012). Moreover, variation in foraging behaviour due to differences in environmental conditions may lead to breeding asynchrony among colonies (Monteiro and Furness, 1998; Jaquemet *et al*., 2008), which has been shown to inhibit gene flow among populations (Smith and Friesen, 2007). For example, allochronic populations of band-rumped storm-petrel *Oceanodroma castro* appear genetically isolated in five archipelagos throughout the Atlantic and Pacific Oceans in the absence of physical barriers to gene flow (Smith and Friesen, 2007). Conversely, whether genetic isolation exists among colonies that exhibit other phenological or circadian differences—e.g. diurnal versus nocturnal colony attendance—has yet to be investigated.

The patchy distribution of suitable habitats for nesting in seabirds (e.g., availability of islands in the oceans) often results in colonies separated by large marine distances (Coulson, 2002), which potentially restricts gene flow among colonies resulting in a positive relationship between marine distance among colonies and genetic divergence. However, Friesen (2007) showed that a simple model of "isolation by distance" provided only a weak explanation of the extent of population genetic structure in seabirds as several species show strong genetic differentiation within single islands or archipelagos. Evidence of intra-specific competition for food suggests greater foraging distances in larger seabird colonies (Lewis *et al*., 2001), implying that population size may affect potential for gene flow. Friesen (2007) found that population genetic structure tended to be less frequent in species with more than 10^6 breeding pairs, which may reflect a relationship with gene flow, but also could be explained by retained ancestral variation (slow drift).

While the overall impact of non-physical (biotic) predictors of genetic structure among seabird colonies has been assessed (Friesen *et al*., 2007), little is known about how these

factors, and the potential interactions between them, contribute to historical processes (e.g. land fragmentation) as explanations for seabird population genetic differentiation. Indeed, studied populations are likely to have experienced bottlenecks, historical fragmentation or otherwise been perturbed during Pleistocene climatic transitions, which can bias the quantification of population genetic structure (e.g., F_{st}, Φ_{st}) (Wright, 1931). Hence, the demographic history of seabird species needs to be considered to discriminate historical from contemporary factors influencing population genetic structure.

Conservation implications for seabirds

Seabirds are more threatened than other group of birds and their status has deteriorated rapidly over recent decades (Croxall *et al*., 2012) with 101 species (29%) currently listed as threatened on the International Union for the Conservation of Nature's (IUCN) Red List of Threatened Species (Spatz *et al*., 2014). Although considerable effort have led to the successful removal of alien species from many islands of substantial importance for breeding seabirds over the last two decades – 333 successful rodent eradications had been undertaken, with invasive rodents eradicated from 284 islands (Howald *et al*., 2007) – the principal threats are still posed by invasive non-native species potentially affecting twice as many seabird species as any other single threat (Schreiber *et al*., 2002; Croxall *et al*., 2012). The remaining threats are evenly divided between those acting mainly at the breeding sites (e.g. human disturbance, infrastructure, commercial and residential development, Spatz *et al*., 2014), those acting mainly at sea in relation to foraging and migrations such as bycatch, pollution and overfishing and the indirect anthropogenic effects such as climatic changes (see review Grémillet and Boulinier, 2009).

To cope with these pressures and adapt to environmental changes – e.g. adapting their foraging effort to buffer the consequences of lower availability of their preferred prey (Litzow *et al*., 2002) – seabirds species specifically need to obtain high levels of genetic diversity. However, compared to other birds, seabird typically exhibit low fecundity and high longevity, which results of their need to prioritize their ability to provide enough food to their offspring especially in unpredictable environment (Lack, 1947), making them unable to respond rapidly to environmental changes. Hence, seabirds are highly vulnerable to extinction if threats to the adult population are persistent rather than episodic. One example is the population decline of the small populations of Galápagos petrel *Spheniscus mendiculus* driving by strong El Niño events of 1982-83 and 1997-98, in the Galápagos Islands (Vargas *et al*., 2007) due to the incapacity of the species to adapt to new environmental conditions. Study showed that the highest values of persistence over the next 100 years were observed in the larger subpopulations of Isabela and Fernandina, which is likely due to both their higher population sizes and more frequent exchange of dispersing birds (Vargas *et al*., 2007).

In addition to the capacity of seabirds to adapt to environmental and anthropogenic changes, connectivity among seabird colonies can increase overall species viability by either allowing recolonization of suitable but unoccupied colonies or re-colonizing locally extinct colonies (Hanski and Gaggiotti, 2004). Indeed, seabirds species breed in discrete, patchy breeding sites located on isolated islands, compared to mainland counterparts, and dispersal among colonies is crucial to maintain the dynamic of such meta-population structures (Coulson, 2002). However, dispersal is considered as flexible trait that may change with time and space depending on environmental conditions – natural and human-mediated shifts (Hanski and Gaggiotti, 2004). Therefore, from a conservation perspective, there is a strong need to

develop predictive models that include changes in connectivity among seabird colonies under different historical and environmental conditions.

1.2 Methodological overview of this thesis

1.2.1 Molecular tools

Genetic markers: screening of loci for PCR amplification success and polymorphism

Molecular tools have become an indispensable part of conservation-based studies (Avise, 1996), providing information ranging from species-level relationships (Krenz *et al*., 2005), cryptic and invasive species recognition (Holland *et al*., 2004), and identification of appropriate source populations for reintroductions (Haig *et al*., 1990). However, despite the broad utility of molecular tools, the combination of genetic markers best suited for any of those particular questions is variable due to different genes often evolving at different rates (Rubinoff, 2006). For example, because it is haploid and maternally inherited, mitochondrial DNA (mtDNA) has an effective population size one-quarter that of the nuclear genome (Ballard and Whitlock, 2004), which makes mtDNA more informative than a single nuclear locus and useful for species-level questions (Rubinoff, 2006). However, its utility is limited by the fact that mitochondrial genes are inherited as a single linkage group and thus cannot provide independent estimates of genetic history (Moore, 1995). In contrast, a set of nuclear genes can be selected from distinct chromosomes, such that they can encapsulate stochastic variation of gene histories (Moore, 1995). As a result, using multiple genetic markers, or loci, is often advocated, even for intra-specific studies, to provide an accurate perspective on an organism's evolutionary history (Funk and Omland, 2003; Ballard and Whitlock, 2004).

Microsatellites or Simple Sequence Repeats (SSRs) have been popular markers in population genetics for the last two decades due to their allelic variability, codominance and high reproducibility of scoring (Distefano *et al*., 2012). However, in many studies about half of candidate loci are rejected for use as a result of insufficient PCR amplification, monomorphism, or multicopy status. While developments in DNA sequencing technology have greatly expedited the discovery of microsatellites (Gardner *et al*., 2011), screening of loci for PCR amplification success and polymorphism remains a costly and time-consuming step (Arthofer *et al*., 2011; Guichoux *et al*., 2011). Recent advances in high-resolution melting (HRM) real-time PCR analysis can potentially expedite this process, reducing both time and monetary costs in comparison to traditional screening involving the use of labelled PCR primers and capillary electrophoresis (CE). While newer techniques exist to survey large numbers of loci (e.g. RAD seq, Davey *et al*., 2013), there is still merit in genotyping microsatellites, particular with respect to their higher allelic diversity. For example, in several fields of research, such as forensics, parentage or kinship studies, microsatellites are preferred to SNPs owing to their higher mutation rates and polyallelic nature (Clayton *et al*., 1998; Vartia *et al*., 2016). Moreover, Haasl (2011) showed that microsatellites offer higher statistical power per locus in the assessment of structure among populations.

A potential issue surrounding the use of HRM to screen polymorphism of microsatellites is that amplicon sizes for HRM should typically be short (80–100 bp) for highest sensitivity (Gundry *et al*., 2008; Herrmann *et al*., 2006; Liew *et al*., 2004; Reed and Wittwer, 2004). However, for microsatellite screening most PCR products will exceed this size owing to optimal placement of primers as inferred from primer design algorithms (e.g. Primer3, Rozen and Skaletsky, 2000), and constraints based on the length and frequency of the repeat motif (e.g. $[AAAG]_{15}$). Furthermore, in downstream analyses there is usually the desire to multiplex

loci during CE, and therefore screen loci with a range of sizes (100–400 bp) (Guichoux *et al*., 2011). In those cases, HRM polymorphism detection decreases and a single dF/dT (derivative of fluorescence over temperature) peak can appear in polymorphic loci, leading to a reduction of sensitivity (1-false negatives; %) of HRM analysis. Previous study on HRM analysis shows that loci with a very narrow melting temperature ranges (ΔT_m) are less likely to be polymorphic (Arthofer *et al*., 2011). However, whether variation in ΔT_m, rather than multiple peaks in dF/dT curves, can better predict polymorphism, has yet to be investigated.

1.2.2 Inferring predictors of genetic differentiation among seabird populations

Testing segregation in non-breeding distributions as a predictor of gene flow by assigning birds to breeding colonies

One of the most important contributions of genetics to conservation is the potential for molecular markers to delimit appropriate population units of management, such as Evolutionary Significant Units (ESUs), defined as populations that do not exchange genes and so are evolutionary independent (Allendorf *et al*., 2010; Moritz, 1994). The identification of ESUs is crucial so that management and monitoring programmes can be efficiently targeted towards distinct or independent populations (Allendorf *et al*., 2010). In practice, the concept of ESUs will complement rather than replace 'species' defined under traditional, predominantly morphological criteria (Moritz, 1994).

One benefit of this concept is the application of methods of 'molecular assignments'. Paetkau *et al*. (1994) demonstrated the possibility of assigning individuals to their population of

origin by comparing individuals' multilocus microsatellite genotype to the allele distributions observed in study populations. In this method, a large number of individuals (> 20) from a number of sites throughout the species' range are typed for one or several loci, generating a baseline of the geographic distribution of alleles. Unknown individuals are typed for the same loci allowing programs (see review Excoffier and Heckel, 2006) to assign a probability to the unknown individuals' membership in each of the baseline populations (Cornuet *et al*., 1999). If the genetic structure among populations is strong, the accuracy for assigning unknown individuals to their natal population is high (Berry *et al*., 2004).

In seabirds, molecular assignments have had several applications including the assessment of anthropogenic mortality, such as fishery bycatch and hunting, which often occurs during the non-breeding season (Friesen, 2007; Walsh and Edwards, 2005). Seabird populations provide a challenge for managers because their geographic ranges can be quite large and they often breed on remote or inaccessible islands (Croxall *et al*., 2012). Hence, the key contributions genetics can make to seabird bycatch are determining the origins and identity of bycatch birds allowing the assessment of extinction risks of populations. For example, a few thousands of black-footed albatross *Phoebastria nigripes* are caught every year in longline fisheries operating in the Gulf of Alaska and in the Bering Sea (Tasker, 2000). The current geographic distribution of the black-footed albatross comprises two main ESUs: the eastern group comprising the northwestern islands of Hawaii, which hold ~95% of the world's population (Walsh and Edwards, 2005), and the western group located off the coast of Japan (Walsh and Edwards, 2005). Walsh *et al*., (2005) used molecular assignments to determine the percentage of the total bycatch that derives from each of its ESUs (100 individuals were tested) and found that only 98% were from the western group, which limits the extinction risks in *P. nigripes* colonies.

Molecular analysis of fisheries' bycatch individuals can test segregation in non-breeding distributions by assigning birds to breeding colonies in addition to telemetry (Edwards *et al*., 2001). Indeed, understanding the relationship between population genetic variation in seabirds and the non-breeding movement patterns of individuals from different colonies requires detailed information on the latter, yet these are often constrained by limited observations. Detailed observations of non-breeding distributions are provided by telemetry studies, but these are typically restricted to a low number of individuals over a relatively short time interval (e.g. a single season), producing temporally and spatially limited insights (Genovart *et al*., 2007). Small rates of gene flow can strongly influence population genetic structure (Slatkin, 1987; Mills and Allendorf, 1996), and therefore foraging observations from a small number of individuals during the non-breeding season may be uninformative about rarer individual movements that can significantly influence genetic variation among colonies. Hence, assuming that genetic structure exists among colonies, this approach has the potential to reject segregation in non-breeding areas in migratory species as a contributor to genetic structure.

Distinguishing historical from biotic processes as explanations for seabird population genetic differentiation

Knowledge derived from genetic analyses of natural populations has been used to advance our understanding of factors influencing gene flow (Wright, 1931; Weir and Cockerham, 1984). However, it is now accepted that the assumptions of models on which several estimators of gene flow were founded are far from realistic (Whitlock and McCauley, 1999; Pearse and Crandall, 2004). Indeed, the demographic history of species can influence estimators of gene flow and needs to be considered to discriminate historical and contemporary factors influencing population genetic structure.

Climatic oscillations during the Pleistocene have shaped the evolution and distribution of species (Hewitt, 1996, 2004). In seabirds, lack of phylogeographic structure has been reported in several Northern Temperate species where populations are suspected to have persisted in refugia over long timescales, such as an entire glacial period (e.g. Moum and Arnason, 2001; Wojczulanis-Jakubas *et al*., 2015). During inter-glacial periods, extensive rapid continued expansions of those populations would have produced considerable genetic homogenization over large areas through gene flow and sequential bottlenecking of peripheral populations during founding (Hewitt, 1996). Therefore, traditional population genetic inferences of contemporary gene flow among those populations are likely to be erroneous (Ibrahim *et al*., 1996).

Physical factors can also lead to population genetic differentiation (Hewitt, 1996). While seabirds are known for their ability to travel long distances, seabirds colonies separated by land show high levels of genetic structure (Friesen, 2015). For example, the Isthmus of Panama, with a minimum width of only ~30 km, prevents dispersal among colonies of Sulids (Steeves *et al*., 2003; Morris-Pocock *et al*., 2010). Similarly, several historical but non-terrestrial barriers have been observed to restrict gene flow among seabird colonies (Friesen, 2015). For example, in Southern Temperate species, poleward shifts of oceanographic fronts caused a split between seabird populations at different latitudes (Younger *et al*., 2016; Munro and Burg, 2017). As a result, although genetic structure also exists in the apparent absence of physical barriers (Burg and Croxall, 2001; Smith and Friesen, 2007; Yeung *et al*., 2009), detecting the impact of historical patterns in seabird differentiation is crucial to distinguish historical versus contemporary processes influencing gene flow among seabird colonies, especially in the context of multi-species comparisons.

Phenotypic adaptations as predictors of genetic isolation among colonies

Phenotypic differences among populations inhabiting different environments may predict their genetic isolation (Avise, 2000). However, in seabirds, differences in morphology and breeding phenology putatively related to environmental differences have not always been accompanied by reciprocal monophyly of mitochondrial lineages (Liebers and Helbig, 2002; Lombal *et al*., 2018), especially at high latitudes (Moum and Arnason, 2001; Liebers and Helbig, 2002). For example, in the lesser black-backed Gull *Larus fuscus*, the split between *L. f. heuglini* and *L. f. fuscus* is reflected in behavioral and ecological segregation, but reciprocal monophyly is lacking for mtDNA (Liebers and Helbig, 2002). This suggests that, in seabirds, recently separated populations may lack population genetic differentiation due to retained ancestral variation (Friesen *et al*., 2007). Conversely, any apparent relationship between phenotypic and genetic divergence also needs to consider potential roles of historical isolation. Therefore, investigating whether differences in breeding phenology and morphology are correlated to genetic structure, and whether any such relationship could be confounded by historical factors, is desirable to accurately detect contributions of contemporary processes to genetic differentiation.

1.2.3 Using ancient DNA to quantify connectivity among populations prior to extinctions

Ancient DNA has revolutionized the field of conservation genetics as specific conservation issues may be informed by the genetic analysis of historic populations (Hofreiter *et al*., 2001; Leonard, 2008; Orlando and Cooper, 2014). Most commonly this involves the reconstitution of past demographic trajectories and testing for coincidence of decline with putative

perturbations, such as the arrival of humans or changes in climate (e.g. Wilmshurst *et al*., 2014; Brüniche-Olsen *et al*., 2018). For example, aDNA study showed that the arrival of humans in northeast Siberia did not affect the mitochondrial diversity of the musk ox *Ovibos moschatus*, one of the few large mammals adapted to a high arctic environment, but rather that both musk ox and humans expanded into Greenland concomitantly (Campos *et al*., 2010). Alternatively, aDNA studies may document cryptic loss of genetic and species diversity (e.g. Calvignac *et al*., 2008; Ramírez *et al*., 2013). For instance, a divergent brown bear *Ursus arctos* mitochondrial DNA lineage not present in any of the previously studied modern or ancient bear samples was identified by Calvignac *et al*., (2008) using aDNA, suggesting that the diversity of *U. arctos* was larger in the past than it is now. In opposition to the approaches described above, the potential of aDNA studies to quantify genetic connectivity among populations prior to extinctions, and to assess its value to offset the processes that have driven these extinctions, has been less commonly realised.

Although few seabird studies to date have used heterochronous' data sets for conservation purposes, which is partly due to the inherent methodological difficulties of aDNA research (Leonard, 2008) and to the scarcity of fossil remains in remote oceanic archipelagos, Welch *et al*. (2012) showed a pattern of dispersal from declining and modern Hawaiian petrel *Pterodroma sandwichensis* colonies by using aDNA methods. Evidence of connectivity, coupled with long generation time, was thus identified as a contributor of genetic stability in this pelagic seabird species despite successive episodes of human colonization during the last 3,000 years. Comparably, Ramírez *et al*., (2013) found that high connectivity buffered genetic diversity in the face of a demographic decline in Cory's shearwaters *Calonectris borealis* colonies using mitochondrial DNA of ancient bone samples from the late-Holocene

together with modern DNA sequences representative of the entire breeding range of the species.

While the ability of high connectivity to buffer genetic diversity in the face of a demographic decline has been reported in several taxa (Jangjoo *et al*., 2016), including marine mammals (Foote *et al*., 2013) which have in common with seabirds the ability to travel long distances, the capacity of connectivity to prevent population extinction also depends on several factors including social, behavioural and demographic characteristics that affect the likelihood of survival and reproduction of migrants (Kleiman, 1989; Short *et al*., 1992). Moreover, while the potential for dispersal will likely help the persistence of colony in the short-term, buffering effects on the genetic diversity may not be enough on a long-term basis if threats affecting the declining of populations exist (see Ramírez *et al*., 2013). Yet, more studies to assess the limits of connectivity to attenuate processes driving population extinctions are desirable.

1.3 Thesis overview

The aims of this thesis are to provide two practical case studies to investigate contemporary mechanisms of genetic differentiation among seabird colonies using DNA analysis based on modern samples for multiple loci in the context of maximizing persistence and resilience of seabird populations. In addition, I analysed subfossils of an extinct population of pelagic seabird to assess whether population extinction occurred in the presence of genetic connectivity with the remaining populations, to assess the limits of genetic connectivity to attenuate processes that have driven extinctions. To investigate how biotic factors (e.g. non-breeding distributions) contribute to historical and physical processes as explanations for

seabird population genetic differentiation, I combined these results with those of over 71 studies testing restrictions in gene flow among seabird colonies.

Chapter 2

Assessment of high-resolution melting (HRM) profiles as predictors of microsatellite variation: an example in providence petrel (Pterodroma solandri).

I employed HRM analysis of 27 microsatellites in providence petrel *Pterodroma solandri*, a pelagic seabird that is IUCN (2012) listed as Vulnerable due to a breeding range restricted to two islands, to examine whether variation in ΔT_m, rather than multiple peaks in dF/dT curves, can better predict polymorphism.

Chapter 3: **Case study #1:** Influence of segregation in non-breeding distributions on genetic differentiation among seabird colonies

Genetic divergence between colonies of flesh-footed shearwater Ardenna carneipes exhibiting different foraging strategies.

The flesh-footed shearwater *Ardenna carneipes* is a species of oceanic seabird listed as vulnerable under the *New South Wales (NSW) Threatened Species Conservation Act* (1995) http://www.legislation.nsw.gov.au/. The species is a trans-equatorial migrant that breeds in northern New-Zealand, Lord Howe Island (Pacific Ocean), on islands off southwestern Australia and Saint-Paul Island (Indian Ocean) (Marchant and Higgins, 1990; Waugh *et al*., 2013; Lavers, 2014), and exhibits high fidelity to natal breeding sites as do most Procellariiformes (Warham, 1990; Brooke, 2004). Geolocation loggers deployed on 61 birds breeding in New Zealand (Rayner *et al*., 2011; Waugh *et al*., 2016) and 57 breeders from

Lord Howe Island (Reid *et al*. 2013) showed that they transit through the central Pacific Ocean to the Sea of Japan for the non-breeding season. Conversely, GPS transmitters deployed on 13 breeders from southwestern Australia (Powell, 2009; Lavers, unpublished data) indicated migration in a north western direction across the southern Indian Ocean to the Arabian Sea. Differences in foraging distribution during the breeding season have also been reported. Individuals breeding east of Australia are believed to forage in more inshore waters (<1000 km from land, Reid *et al*., 2012) and at a higher trophic level than individuals breeding on islands in Western Australia (Lindsey, 1986; Taylor, 2000; Bond and Lavers, 2014).

I generated a dataset of DNA sequences from one mitochondrial region and seven nuclear loci to test the hypothesis that eastern and western *A. carneipes* breeding colonies form two independent genetic clusters, as suggested by observed high philopatry and evidence of different foraging distributions and strategies during the breeding and post-breeding season. However, to more rigorously test the assumption of segregation in non-breeding distributions as a predictor of genetic structure among colonies, I inspected mtDNA sequences from fisheries' bycatch individuals obtained in Japanese waters in the North Pacific Ocean.

Chapter 4: **Case study #2**: Influence of circadian differences– e.g. diurnal versus nocturnal – between colonies on genetic differentiation

Population genetic and behavioural variation of the two remaining colonies of providence petrel (Pterodroma solandri).

The providence petrel (*Pterodroma solandri*) is classified as vulnerable under both the *IUCN Red List of Threatened Animals* (Criteria D2) and the *New South Wales Threatened Species*

Conservation Act 1995 due to its restricted breeding range. The only significant breeding locality is Lord Howe Island (~32,000 breeding pairs) (Bester, 2003), a small island located 600 km off the eastern coast of Australia. Providence petrels previously bred on Norfolk Island (~1,000,000 breeding pairs), located approximately 1100 km northeast of Lord Howe Island, before becoming extirpated following European settlement by the late 18th century (Medway, 2002a). This species was considered extinct within the Norfolk Island group until 1986 when a small population (~20 breeding pairs) was discovered on Phillip Island, 7 km south of Norfolk Island (Hermes *et al*., 1986).

There is no evidence justifying taxonomic separation between Phillip Island and Lord Howe Island providence petrels. However, it has been reported that Lord Howe Island individuals predominantly arrive at the colony during daylight (Bester *et al*., 2002), while Phillip Island individuals return to their breeding sites only after dusk (pers. obs.). This may relate to the historical presence of diurnal aerial predators – Brown Goshawks *Accipiter fasciatus* – on Norfolk Island (Medway, 2002b), although no such predation risk presently exists. Alternatively, differences in foraging areas may explain time of return to colony (e.g. Dias *et al*., 2012). Given the possibility of selective significance, the observed difference in behaviour between colonies may inhibit gene flow between them.

In Chapter 4, I report a comprehensive study of the genetic distinctiveness between the two remaining breeding colonies of providence petrel, to infer the dispersal patterns of this species given the difference in behaviour (diurnal versus nocturnal) reported between them. I developed three genetic data sets, consisting of DNA sequences from mitochondrial and 14 nuclear regions and genotypes from 10 microsatellite loci, to investigate genetic connectivity and evolutionary history of providence petrel colonies. This study is also relevant to the

proposed re-establishment of a colony on Norfolk Island using individuals from Lord Howe Island, with the aim of reducing the extinction risk of this species, and restoring the input of marine-derived nutrients into the Norfolk Island ecosystem, as any genetic novelty of the Phillip Island population could be at risk.

Chapter 5: **U**sing ancient DNA methods to assess genetic connectivity of the extinct Norfolk Island colony and extant colonies of providence petrels *Pterodroma solandri*

Ancient DNA reveals population extinction despite genetic connectivity in the providence petrel (Pterodroma solandri).

While a detailed study of the genetic distinctiveness between the two extant breeding colonies using three genetic data sets was conducted in the previous chapter, it is not known whether the Norfolk Island colony went extinct while genetically connected with other colonies. Furthermore, it is not known whether this extinction may reflect cryptic loss of species diversity (see review Ramakrishnan and Hadly, 2009). Moreover, information on past connectivity between providence petrel colonies may inform whether the nocturnal behaviour observed for Phillip Island individuals reflects an ancestral adaptation to diurnal predators on Norfolk Island, or a recent adaptation to new environmental conditions.

I used ancient DNA methods to compare mitochondrial Cytochrome *b* sequences from Norfolk Island subfossil remains to those of modern providence petrels. My goal was to quantify whether the Norfolk Island colony declined in the presence of genetic connectivity from other populations, which is essential to assess the limits of genetic connectivity to attenuate processes that have driven extinctions. Secondly, I tested whether previously

unrecognized taxonomic or genetic distinctiveness was lost during the Norfolk Island extinction, and whether the nocturnal behaviour of contemporary Phillip Island individuals reflects a genetic legacy of past connection to the Norfolk Island population.

Chapter 6 Multi-species analysis inferring predictors of genetic differentiation among seabird populations

Historical and physical factors dominate biotic processes as determinants of seabird population genetic differentiation

I conducted multiple generalized linear models (GLMs) based on mtDNA variation to investigate factors influencing genetic differentiation among seabird colonies. To identify historical contributors to genetic divergence, I investigated i) the overall impact of demographic history (e.g. expansion from refugia during postglacial periods) on genetic structure among seabird colonies, and ii) the impact of physical barriers on genetic structure among colonies. To identify biotic (e.g. contemporary) contributors to population divergence, I investigated whether factors such as the segregation in non-breeding distributions predict genetic structure among colonies. In addition, I investigated whether differences in breeding phenology and morphology are correlated to genetic structure, and whether any such relationship could be confounded by historical factors.

Chapter 7

General discussion and concluding remarks

This concluding chapter consolidates my most significant findings. I specifically identify interpretation where caution is needed and improvements could be made, particularly with

regard to the methodology used to identify the processes of genetic differentiation among populations. Finally, I provide future direction and objectives of conservation genetics as we enter the era of high throughput sequencing.

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Chapter 2:

Assessment of high-resolution melting (HRM) profiles as predictors of microsatellite variation - An example in providence petrel (*Pterodroma solandri*)

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Assessment of high-resolution melting (HRM) profiles as predictors of microsatellite variation - An example in providence petrel (*Pterodroma solandri*)

Anicee J. Lombal[1,2], Theodore J. Wenner[1,3] and Christopher P. Burridge[1,4].

[1]School of Biological Sciences, University of Tasmania, Private Bag 55, Hobart, TAS 7001, Australia. Fax: +61 3 6226 2745, Phone: +61 3 6226 7653.

[2] Anicee.Lombal@utas.edu.au

[3] Theodore.Wenner@utas.edu.au

[4] Chris.Burridge@utas.edu.au

Abstract

High-resolution melting (HRM) analysis is an emerging technology to screen microsatellites for polymorphism. A potential issue surrounding this method is that amplicon sizes for HRM should typically be short (80–100 bp) for highest sensitivity to reveal polymorphism via the presence of two peaks in the curve of the derivative of fluorescence over temperature (dF/dT). In contrast, microsatellite amplicons are typically 100–400 bp. Therefore, I compared HRM analysis melting temperature range (ΔT_m) and multiple dF/dT peaks for predicting microsatellite polymorphism. I assessed polymorphism at 27 microsatellite loci, with estimated lengths of 122–321 bp, in providence petrel (*Pterodroma solandri*). I validated HRM assessment using traditional capillary electrophoresis (CE). While 100% of loci exhibiting multiple peaks in the dF/dT curve were confirmed as polymorphic by CE, 16% improvement in sensitivity (83% versus 67%) was achieved by using ΔT_m, and 25% (92% versus 67%) by using ΔT_m in addition to multiple dF/dT peaks. I suggest HRM melting temperature range as new predictor of polymorphism that can be used to rapidly assess microsatellites polymorphism.

Keywords: high-resolution melting analysis, microsatellite, polymorphism, melting temperature range, *Pterodroma solandri*

Introduction

Microsatellites or Simple Sequence Repeats (SSRs) have been popular markers in population genetics for the last two decades due to their allelic variability, codominance and high reproducibility of scoring (Distefano *et al*., 2012). However, in many studies about half of candidate loci are rejected for use as a result of insufficient PCR amplification, monomorphism, or multicopy status. While developments in DNA sequencing technology have greatly expedited the discovery of microsatellites (Gardner *et al*., 2011), screening of loci for PCR amplification success and polymorphism remains a costly and time-consuming step (Arthofer *et al*., 2011; Guichoux *et al*., 2011). Recent advances in high-resolution melting (HRM) real-time PCR analysis can potentially expedite this process, reducing both time and monetary costs in comparison to traditional screening involving the use of labeled PCR primers and capillary electrophoresis (CE).

HRM is a closed-tube method based on PCR amplification in the presence of a saturating dye, e.g. EvaGreen, followed by a high-resolution melting step (Liew *et al*., 2004; Reed and Wittwer, 2004). During the melting step, changes in the strength of fluorescence signal are recorded as the double-stranded DNA disassociates. This transition is a function of amplicon length and nucleotide composition (i.e. % of GC content), and is represented by peaks in the curve of the derivative of fluorescence over temperature (dF/dT, Tindall *et al*., 2009). In theory, heterozygous individuals will produce a dF/dT curve containing two peaks; heteroduplex molecules will have a lower melting temperature (T_m), reflecting nucleotide mispairing in the double stranded molecule, resulting in an early peak in the dF/dT curve relative to the two possible homoduplex molecules, that are usually indistinguishable from each other (Figure 2 – 1). More than two dF/dT peaks within an individual may represent

multicopy loci, multiple mutated sites, or amplification of non-specific bands, while the absence of dF/dT peaks indicates insufficient amplification (Arthofer *et al*., 2011).

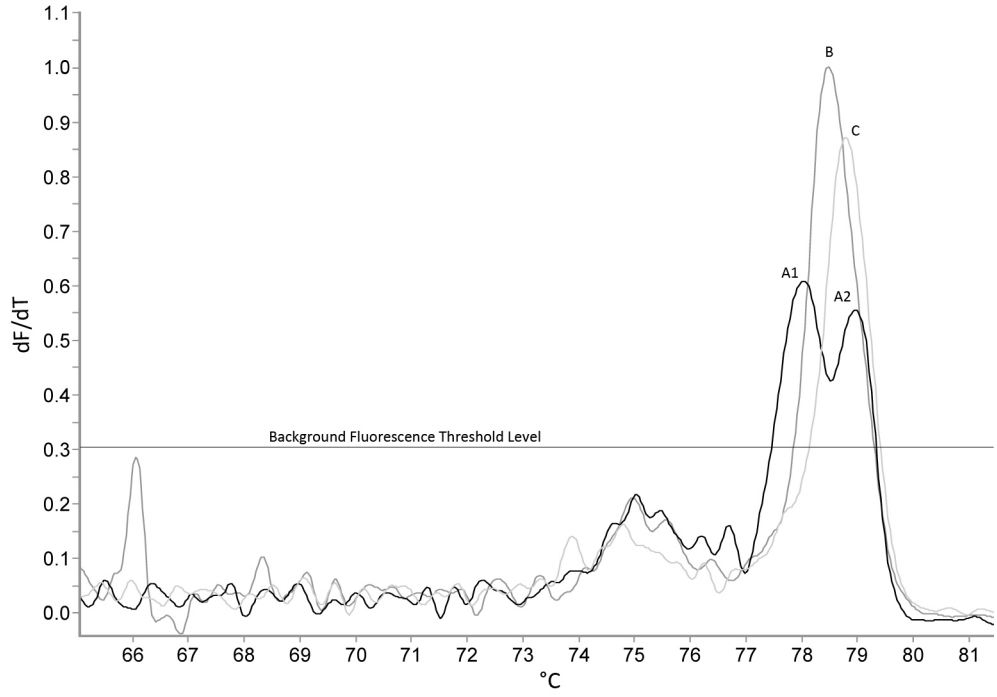

Figure 2 – 1 dF/dT curve of locus *Ptero09* showing a heterozygous individual (black) exhibiting typical double peaks (black, A1, A2) and homozygous individuals (grey, B, C) showing single peaks. Threshold is adjusted above background noise.

A potential issue surrounding the use of HRM to screen polymorphism of microsatellites is that amplicon sizes for HRM should typically be short (80–100 bp) for highest sensitivity (Liew *et al*., 2004; Reed and Wittwer, 2004; Herrmann *et al*., 2006; Gundry *et al*., 2008). However, for microsatellite screening most PCR products will exceed this size owing to optimal placement of primers as inferred from primer design algorithms (e.g. Primer3) (Rozen and Skaletsky, 2000), and constraints based on the length and frequency of the repeat motif (e.g. $[AAAG]_{15}$). Furthermore, in downstream analyses there is usually the desire to multiplex loci during CE, and therefore screen loci with a range of sizes (100–400 bp) (Guichoux *et al*., 2011). In those cases, HRM polymorphism detection decreases and a single dF/dT peak can appear in polymorphic loci, leading to a reduction of sensitivity (1-false negatives; %) of HRM analysis.

Previous study on HRM analysis shows that loci with a very narrow melting temperature ranges (ΔT_m) are less likely to be polymorphic (Arthofer *et al*., 2011). Here, I investigate whether variation in ΔT_m, rather than multiple peaks in dF/dT curves, can better predict polymorphism. Given the negative relationship between ΔT_m and fragment length (Liew *et al*., 2004; Gundry *et al*., 2008; Muleo *et al*., 2009; Smith *et al*., 2010; Arthofer *et al*., 2011), I also examined the effect of fragment length on polymorphism to verify that any relationship between ΔT_m and polymorphism does not simply reflect spurious correlation owing to a relationship between repeat number (and therefore fragment length) and polymorphism (Brinkmann *et al*., 1998; Bachtrog *et al*., 2000).

To examine these hypotheses I assessed polymorphism of 27 microsatellites using HRM in providence petrel (*Pterodroma solandri*) (Table 2 – 1), a pelagic seabird that is IUCN listed as Vulnerable due to a breeding range restricted to two islands (IUCN 2012). The loci

screened have been developed from other members of Procellariiform (Table 2 – 1), and both amplification success and polymorphism are less likely in providence petrel in comparison to loci characterized directly from the target species (Crawford *et al*., 1998). In addition, there is no sequence information from providence petrel with which to redesign primers and reduce amplicon size in an effort to increase HRM sensitivity. Under these circumstances a rapid and low-cost (no requirement of labeled primers or electrophoresis) method to assess polymorphism is particularly desirable.

Table 2 – 1 Results of high-resolution melt (HRM) analysis and capillary electrophoresis (CE) at 27 microsatellite loci in 10 *Pterodroma solandri* individuals (five individuals from Phillip Island and five individuals from Lord Howe Island). [a]Dubois *et al*., 2005; [b]Küpper *et al*., 2007; [c]Burg, 1999; [d]Sun *et al*., 2009; [e]Techow and O'Ryan, 2004; [f]Brown and Jordan, 2009; [g]Welch and Fleischer, 2011; [h]Given *et al*., 2002.

Microsatellite Loci Background Information			HRM Screening		CE	
Loci	Published Size Range	Originally Reported Repeat Motif	Max dF/dT Peaks	ΔT_m (°C)	Size (bp)	Number of Alleles
10C5[a]	160-163	$(GA)_{11}(GC)_2GT(GC)_2$	1	1.55	175	1
12H8[a]	185-191	$(GT)_7(AT)_7$	>2	-	-	-
Calex01[b]	224-232	$(GT)_2GA(GT)_3GC$	2	2.07	237-255	7
De11[c]	164-170	$(AC)_{9.5}(TA)_7(CA)_4(CG)_5$	1	0.68	186	1
OC84[d]	317-321	$(AG)_9$	1	1.25	315	1
Oc87B[d]	280-286	$(GA)_{12}$	>2	-	-	-
Paequ3[e]	228-262	$(GA)_{19}$	2	0.98	222-232	5
Paequ10[e]	152-154	$(CA)_8$	1	0.48	204	1
Paequ13[e]	136-142	$(GT)_9$	2	1.47	146-148	2
Parm01[f]	201-235	$(CA)_{13}$	1	0.61	159-230	6
Parm02[f]	179-200	$(CA)_6TA(CA)_9$	2	2.13	192-198	3
Parm03[f]	174-192	$(CA)_5TA(CA)_{11}$	1	1.30	177-181	3
Parm04[f]	207	$(CA)_5(GACA)_2$	>2	-	-	-
Parm05[f]	122	$(CA)_{11}$	1	0.67	144	1
Parm06[f]	160	$(CA)_6GA(CA)_2TACA$	1	0.80	182	1
Ptero01[g]	163-167	$(CA)_7$	1	1.47	187	1
Ptero02[g]	131-141	$(CA)_9$	1	0.38	136	1
Ptero03[g]	130-142	$(CA)_9$	1	0.66	157	1
Ptero04[g]	146-160	$(CA)_{13}$	2	5.40	150-168	5
Ptero05[g]	206-215	$(AAG)_2AGG(AAG)_3$	1	1.00	235	1
Ptero06[g]	145-177	$(AAGG)_{13}$	1	1.85	141-149	3
Ptero07[g]	253-289	$(AAAG)_8$	2	1.93	264-344	16
Ptero08[g]	162-226	$(AAGG)_8$	>2	-	-	-
Ptero09[g]	212-236	$(AAGG)_8$	2	1.35	187-235	10
Ptero10[g]	205-290	$(TAGGA)_9\ldots(TAGGA)_7$	>2	-	-	-
RBG18[h]	145-159	$(GT)_{11}$	1	1.07	196-199	2
RBG29[h]	155-163	$(GT)_{13}$	2	1.15	124-136	5

Materials and Methods

PCR amplification and High Resolution Melting (HRM) analysis

HRM was performed on a Rotorgene Q (Qiagen, Valencia, CA USA) using five individuals from both of the two known *P. solandri* populations (Lord Howe Island, 31°30'S, 159°05'E; Phillip Island, 29°12'S, 167°95'E). Reactions consisted of 1X Type-it HRM PCR kit (Qiagen), 0.175 μM forward and reverse primers, and 0.8 μL template DNA (ca. 10 ng/μL) in 10 μL total reaction volume. Cycling conditions were 3 min initial hot start at 95°C followed by 70 cycles of 95°C for 10 s, 55°C for 15 s, and 72°C for 20 s, with amplification success quantified by fluorescence acquired after each elongation step. Cycling was followed by a 1 min hold at 95°C, and then rapid cooling to a 40°C hold for 1 min, to ensure complete renaturation of products and to maximize heteroduplex formation (Smith *et al*., 2010). Melting curves were generated using continuous fluorescence consisting of a 65°C hold for 1 min, followed by ramping to 90°C in 0.1°C increments with fluorescence acquired after holding for 2 s at each increment. Comparative quantitation and melting-curve analysis were performed using the Rotor-Gene Q Series Software v2.0.2 (Qiagen). A derivative plot of the probe melting region showing the negative derivative of normalized fluorescence with respect to temperature (dF/dT) was determined by Solvitsky-Golay polynomial estimation (Wittwer and Kusukawa, 2003), where peak amplitudes indicate temperature point of maximum amplicon dissociation (ΔT_m) (Figure 2 – 1). For each marker, the following parameters were recorded: (1) the number of peaks of each individual curve where the dF/dT threshold was manually adjusted above background noise in the exponential phase of the run (Figure 2 – 1). (2) the melting transitions of each peak for parameter (1) given initially by automatic ΔT_mcalculation under 'SYBR Green I Format' where the number of 'Maximal peaks' was set at '2 or less' by default (Table 2 – 1). I assumed that the ΔT_mvalues were normally distributed, that the estimated means were representative of the true population

means, and that the variation was equal and representative for both populations. I excluded loci showing complex melting patterns (>2 dF/dT peaks in an individual) given these primers may be amplifying multiple loci, although they could also represent single loci with multiple melt regions and multiple SNPs (Wu *et al*., 2008).

Capillary Electrophoresis (CE)

All amplicons from loci showing one or two peaks in the dF/dT curve were subjected to CE. Forward primers were dye labeled with either NED, PET, VIC, or FAM and amplified in three multiplex reactions where each label type was represented for only a single locus, employing the MyTaq HS (Bioline) protocol. Products were then separated by CE on a AB3730*xl* DNA Analyzer (Applied Biosystems Inc.) using the LIZ600 size standard.

Statistical analyses

I compared means of ΔT_m and fragment length for polymorphic and monomorphic loci using a one-way between groups ANOVA (parametric distribution) and a Wilcox-test (non-parametric distribution), respectively. I constructed kernel density estimation of both ΔT_m and fragment length with respect to polymorphism using the package *sm* in R version 3.1.2 (Everitt and Hothorn, 2003), and assessed the junction of their distributions. I analyzed the variation in sensitivity (1-false negatives, %) versus specificity (1-false positives, %) of HRM analysis according to ΔT_m with the package *ggplot2* in R. I detected a threshold maximizing both specificity and sensitivity of HRM analysis and I compared these results to those obtained when using the presence of two peaks in the dF/dT curve to assess polymorphism.

Results

Five loci exhibiting more than two peaks in the dF/dT curve for an individual were discarded. Eight loci exhibiting two dF/dT peaks for at least one individual were confirmed as polymorphic with CE, displaying between two and 16 alleles. Four loci failed to be detected as polymorphic, showing only a single dF/dT peak (sensitivity = 1-False negative = (1-4/12) = 67%). All monomorphic loci exhibited single dF/dT peaks (specificity = 1-False positive = 1-(0/10) = 100%) (Table 2 – 1). ΔT_m for *Ptero04* deviated substantially from the distribution of other loci, and was excluded as an outlier for statistical analysis (Table 2 – 1). The difference between means of ΔT_m for polymorphic and monomorphic loci was significant (ANOVA, $F = 7.45$, $p = 0.013$), but the difference between means of sequence lengths for polymorphic and monomorphic loci was not (Wilcox-test, $p = 0.459$) (Figure 2 – 2). For this reason, I pursued analyses of ΔT_m. The junction between the two probability density functions of ΔT_m with respect to polymorphism was estimated as ~1.0°C (Figure 2 – 3). I then detected 1.07°C as the optimal ΔT_m to maximize both sensitivity (83%) and specificity (70%) (Figure 2 – 4; Table 2 – 2). A significant improvement in sensitivity (1-(2/12) = 83% versus 1-(4/12) = 67%) was achieved by using ΔT_m (threshold 1.07°C) to assess polymorphism relative to multiple peaks in the dF/dT curve for an individual (Table 2 – 2). A larger improvement in sensitivity (1-(1/12) = 92% versus 1-(4/12) 67%) was achieved by using this ΔT_m threshold in addition to multiple peaks in the dF/dT curve to assess polymorphism (Table 2 – 2).

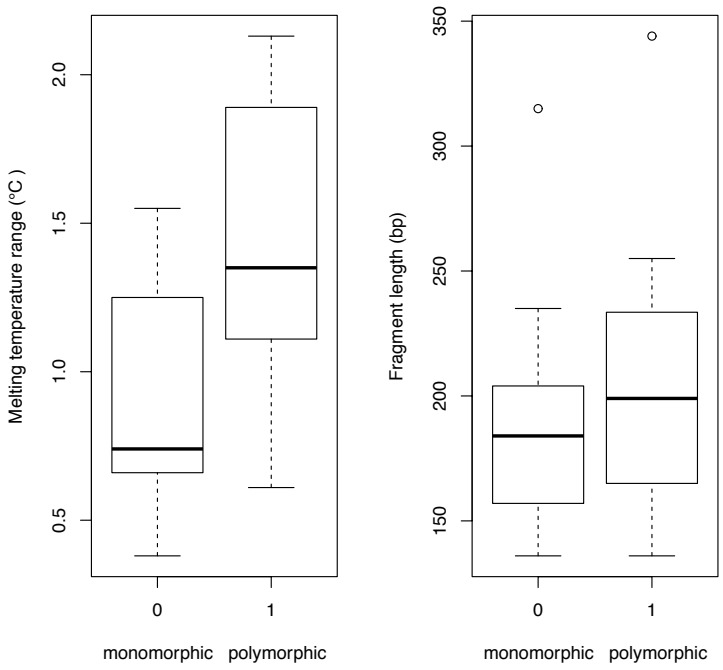

Figure 2 – 2 Boxplots describing melting temperature range (ΔT_m) and fragment length with respect to polymorphism in 22 microsatellite loci from *Pterodroma solandri*. Left, means of ΔT_m with respect to monomorphic (0.84°C) and polymorphic (1.40°C) loci. Right, means of fragment length with respect to monomorphic (192 bp) and polymorphic (209 bp) loci.

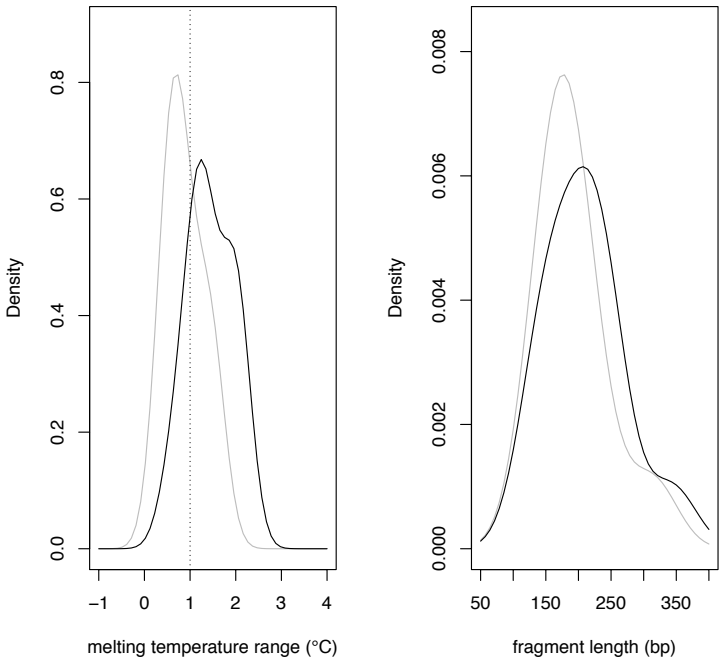

Figure 2 – 3 Kernel density estimations showing the probability density functions of high resolution melt temperature range (ΔT_m) and fragment length based on their repartition with respect to polymorphism. Left, ΔT_m. Right, fragment length. Grey lines represent probability density functions of monomorphic loci; black lines represent probability density functions of polymorphic loci. Dotted lines represent an assessment of the junction between monomorphic and polymorphic probability density functions.

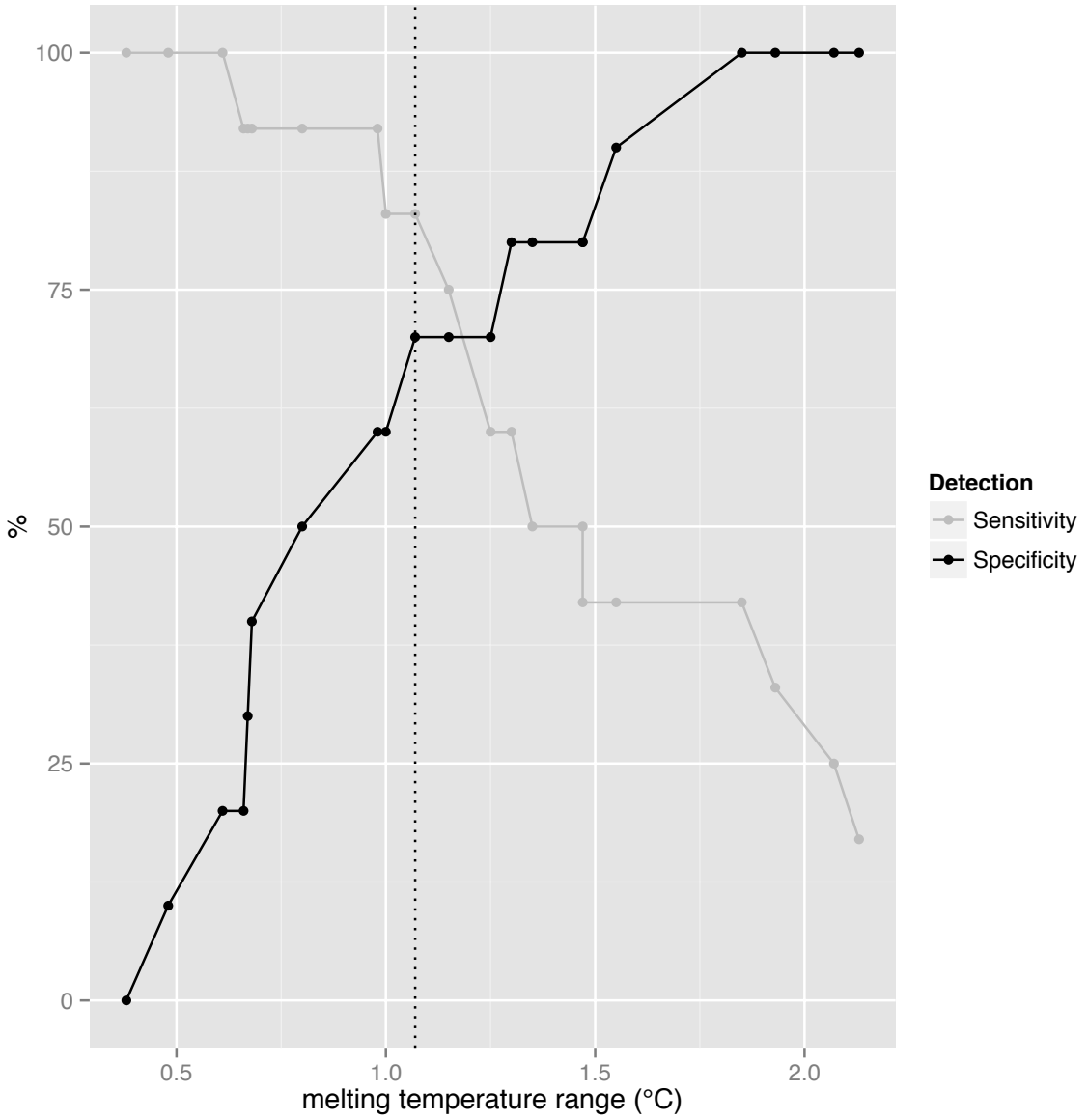

Figure 2 – 4 Variation of high resolution melt detection (%) according to melting temperature range (ΔT_m). Grey line represents sensitivity (1-false negatives; %); black line represents specificity (1-false positives; %). Dotted line represents the optimal detection threshold ΔT_m.

Table 2 – 2 Results of high resolution melt detection of polymorphism at 22 microsatellite loci in 10 *Pterodroma solandri* individuals using different predictors.

Detection of polymorphism with dF/dT peaks within an individual, melting temperature range (ΔT_m), or dF/dT peaks and ΔT_m (right detection = +; wrong detection = –). Sensitivity (1-false negatives) and specificity (1-false positives) are represented as percentages.

					HRM inference of polymorphism		
Loci	Alleles	Size (bp)	ΔT_m	Poly. Y/ N	dF/dT peaks	ΔT_m (>1.07)	dF/dT peaks + ΔT_m
Ptero02	1	136	0.38	N	+	+	+
Paequ10	1	204	0.48	N	+	+	+
Parm01	6	230	0.61	Y	–	–	–
Ptero03	1	157	0.66	N	+	+	+
Parm05	1	144	0.67	N	+	+	+
De11	1	186	0.68	N	+	+	+
Parm06	1	182	0.8	N	+	+	+
Paequ3	5	232	0.98	Y	+	–	+
Ptero05	1	235	1	N	+	+	+
RBG18	2	199	1.07	Y	–	+	+
RBG29	5	136	1.15	Y	+	+	+
Oc84	1	315	1.25	N	+	–	+
Parm03	3	181	1.3	Y	–	+	+
Ptero09	10	235	1.35	Y	+	+	+
Ptero01	1	187	1.47	N	+	–	+
Paequ13	2	148	1.47	Y	+	+	+
10C5	1	175	1.55	N	+	–	+
Ptero06	3	149	1.85	Y	–	+	+
Ptero07	16	344	1.93	Y	+	+	+
Calex01	7	255	2.07	Y	+	+	+
Parm02	3	198	2.13	Y	+	+	+
Ptero04	5	168	5.40	Y	+	+	+
Sensitivity (%)					67	83	92
Specificity (%)					100	70	100

Discussion

Sensitivity and specificity of HRM analysis depend mainly on instrument resolution and amplicon size (Herrmann *et al*., 2006). Here, I assessed polymorphism at 27 microsatellite loci, with lengths of 122–321 bp, and I showed that ΔT_m better predicted polymorphism than fragment length or multiple dF/dT peaks for an individual. I also detected a threshold value of ΔT_m to assess polymorphism that can be generalized to further studies. Nevertheless, as reaction conditions can widely vary between PCR products, the reproducibility of T_m measurements can be compromised (e.g. T_m standard deviation of 0.03°C to 0.39°C attributable to DNA extractions already observed). To partly correct resolution limitations imposed by the instrument and solution chemistry between samples, internal temperature controls (or complementary unlabeled oligonucleotides) can be included, allowing subsequent temperature correction of the melting profile, leading to a reduction of T_m standard deviation (Seipp *et al*., 2007). This will not control for concentration of amplified DNA, but since different PCR reactions tend to plateau at the same product concentration, these variations represent a minor concern (Gundry *et al*., 2003).

Regardless of the decrease of specificity (increase in false positives) observed by using HRM ΔT_m to assess polymorphism relative to multiple dF/dT curves, I suggest ΔT_m as an additional predictor that can detect more polymorphic loci for subsequent analysis. HRM is substantially cheaper and faster than CE, even when employing cost-efficient methods of dye label incorporation (Schuelke, 2000; Arthofer *et al*., 2011), as the HRM approach does not have electrophoresis costs. HRM also outperforms significantly conventional PCR and CE in terms of processing time (Arthofer *et al*., 2011). Other approaches to screen polymorphism that avoid labeled primers, such as high-resolution agarose (Hughes and Queller, 1993) or non-denaturing polyacrylamide electrophoresis (Lessa and Applebaum, 1993) also require

electrophoresis relative to HRM, and likewise exhibit low sensitivity relative to denaturing CE (Andersen *et al*., 2003). Furthermore, in contrast to CE, HRM thermal cyclers will also become increasingly available within laboratories as their ability to perform real-time genotyping of single loci become increasingly realized (MacKay *et al*., 2008; Muleo *et al*., 2009; Smith *et al*., 2010; Dang *et al*., 2012).

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Chapter 3:

Genetic divergence between colonies of flesh-footed shearwater *Ardenna carneipes* exhibiting different foraging strategies

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Genetic divergence between colonies of flesh-footed shearwater *Ardenna carneipes* exhibiting different foraging strategies

Anicee J. Lombal[1,5], Theodore J. Wenner[1,6], Jennifer L. Lavers[2,7], Jeremy J. Austin[3,8], Eric J. Woehler[2,9], Ian Hutton[4,10], and Christopher P. Burridge[1,11].

[1]School of Biological Sciences, University of Tasmania, Hobart, TAS 7001, Australia.

[2]Institute for Marine and Antarctic Studies, University of Tasmania, Hobart, TAS 7004, Australia.

[3]Australian Centre for Ancient DNA, School of Biological Sciences, University of Adelaide, Adelaide, SA 5005, Australia.

[4]Lord Howe Island Museum, PO Box 157, NSW 2898, Australia.

[5]Anicee.lombal@utas.edu.au

[6]Theodore.wenner@utas.edu.au

[7]Jennifer.lavers@utas.edu.au

[8]Jeremy.austin@adelaide.edu.au

[9]Eric.woehler@utas.edu.au

[10]Ian@ianhuttontours.com

[11]Chris.burridge@utas.edu.au

Keywords: oceanic seabirds, *Ardenna carneipes*, gene flow, genetic divergence, foraging segregation, genetic assignment, conservation management

Abstract

Increasing evidence suggests foraging segregation as a key mechanism promoting genetic divergence within seabird species. However, testing for a relationship between population genetic structure and foraging movements among seabird colonies can be challenging. Telemetry studies suggest that flesh-footed shearwater *Ardenna carneipes* that breed at Lord Howe Island or New Zealand, versus southwestern Australia or Saint-Paul Island in the Indian Ocean, migrate to different regions (North Pacific Ocean and northern Indian Ocean, respectively) during the non-breeding season, which may inhibit gene flow among colonies. In this study, I sequenced a 858-base pair mitochondrial region and seven nuclear DNA fragments (352–654 bp) for 148 individuals to test genetic differentiation among colonies of flesh-footed shearwaters. Strong genetic divergence was detected between Pacific colonies relative to those further West. Molecular analysis of fisheries' bycatch individuals sampled in the Sea of Japan indicated that individuals from both western and eastern colonies were migrating through this area, and hence the apparent segregation of the non-breeding distribution based on telemetry do not corroborate genetic data. The genetic divergence among colonies is better explained by philopatry and evidence of differences in foraging strategies during the breeding season, as supported by the observed genetic divergence between Lord Howe Island and New Zealand colonies. I suggest molecular analysis of fisheries' bycatch individuals as a rigorous method to identify foraging segregation, and I recommend the eastern and western *A. carneipes* colonies be regarded as different Management Units.

Introduction

Understanding evolutionary processes and population dynamics within species is crucial to predict their long-term persistence and resilience to environmental perturbations (Avise and Hamrick, 1996). This requires investigating gene flow among populations to assess local extinction risk (Wright, 1931; Chepko-Sade and Halpin, 1987; Ibrahim *et al*., 1996). Isolation of populations can lead to genetic divergence, and often a decrease in genetic diversity through stochastic events such as genetic drift and increased inbreeding in small populations (Moritz, 1999; Frankham, 2010). This may increase extinction risk by reducing the potential for adaptation to future changes such as environmental variations and anthropogenic stressors, although gene flow can also inhibit adaptation by swamping favoured alleles (Frankham, 2005). Consequently, predicting gene flow between populations based on factors such as wind-dispersed seeds in plants (Hamrick *et al*., 1992) or presence of pelagic larvae versus direct development in fishes (Kyle and Boulding, 2000; Dawson *et al*., 2014) is highly desirable for identifying conservation priorities and maintaining viability of species (Greenwood *et al*., 1978; DeSalle and Amato, 2004).

Among seabirds, several non-physical factors are associated with restricted movement and spatial structuring of genetic variation among colonies (Friesen *et al*., 2007). Although most seabird species have the ability to travel long distances (Shaffer *et al*., 2006), they also usually exhibit a high level of philopatry (Warham, 1990; Coulson, 2002), which appears to restrict gene flow among colonies in some species (Friesen *et al*., 2007; Friesen, 2015). However, a number of seabird species showing philopatry do not present genetic structure among colonies (e.g. Austin *et al*., 1994; Avise *et al*., 1992; Pearce *et al*., 2004; Roeder *et al*., 2001), such that philopatry is not always a predictor of population genetic structure or has not been accurately quantified in those species. Seabirds from different colonies also often

display discrete foraging distributions during breeding or non-breeding seasons that may limit gene flow among populations and promote local differentiation (Catard *et al*., 2000; Peck and Congdon, 2005).

Some seabirds that migrate to population-specific non-breeding areas appear to have less opportunity for gene flow among populations than those that have a single common non-breeding area (Burg and Croxall, 2001; Friesen *et al*., 2007; Kidd and Friesen, 1998). For example, Burg and Croxall (2001) found that black-browed albatrosses *Thalassarche* spp. showing distinct foraging grounds during the non-breeding season differ genetically despite a lack of physical barriers to dispersal among colonies. However, segregation during the non-breeding season *per se* is unlikely to always explain restrictions in gene flow among seabird colonies (Rayner *et al*., 2011a), as 63% of species whose populations overlap in non-breeding distribution show evidence of restrictions in gene flow among colonies (Friesen, 2015). Population-specific foraging distribution during the breeding season may also restrict gene flow among colonies (Friesen *et al*., 2007; Wiley *et al*., 2012). Hawaiian Petrels *Pterodroma sandwichensis* nesting on Hawaii versus Kauai and foraging in different areas during the breeding season exhibit significant spatial genetic structure (Wiley *et al*., 2012). However, a greater number of studies are required to test whether differences in non-breeding foraging distributions influence genetic divergence between populations, and to provide insights into behavioural and ecological mechanisms underlying the population genetic diversification of highly mobile taxa, such as seabirds.

Understanding the relationship between population genetic variation in seabirds and the foraging distributions of individuals from different colonies requires detailed information on the latter, yet these are often constrained by limited observations. Detailed observations of

foraging movements are provided by telemetry studies, but these are typically restricted to a low number of individuals over a relatively short time interval (e.g. a single season), producing temporally and spatially limited insights at best (Genovart *et al*., 2007). Small rates of gene flow can strongly influence population genetic structure (Slatkin, 1987; Mills and Allendorf, 1996), and therefore foraging observations from a small number of individuals may be uninformative about rarer individual movements that can significantly influence genetic variation among colonies. In addition to telemetry, molecular analysis of fisheries' bycatch individuals can test foraging segregation by assigning birds to breeding colonies (Edwards *et al*., 2001) assuming that genetic structure exists among colonies; this approach has the potential to reject foraging segregation as a contributor to genetic structure.

The flesh-footed shearwater *Ardenna carneipes* is a species of oceanic seabird listed as vulnerable under the *New South Wales (NSW) Threatened Species Conservation Act* (1995) http://www.legislation.nsw.gov.au/. The species is a trans-equatorial migrant that breeds in northern New-Zealand, Lord Howe Island (Pacific Ocean), on islands off southwestern Australia and Saint-Paul Island (Indian Ocean) (Marchant and Higgins, 1990; Waugh *et al*., 2013; Lavers, 2014), and exhibits high fidelity to natal breeding sites as do most Procellariiformes (Warham, 1990; Brooke, 2004). Geolocation loggers deployed on 61 birds breeding in New Zealand (Rayner *et al*., 2011b; Waugh *et al*., 2016) and 57 breeders from Lord Howe Island (Reid *et al*., 2013; Tuck and Wilcox, 2010) showed that they transit through the central Pacific Ocean to the Sea of Japan for the non-breeding season. Conversely, GPS transmitters deployed on 13 breeders from southwestern Australia (Powell, 2009; Lavers, unpublished data) indicated migration in a north-western direction across the southern Indian Ocean to the Arabian Sea. Differences in foraging distribution during the breeding season have also been reported. Individuals breeding east of Australia are believed

to forage in more inshore waters (<1000 km from land, Reid *et al*., 2012) and at a higher trophic level than individuals breeding on islands in Western Australia (Lindsey, 1986; Taylor, 2000; Bond and Lavers, 2014).

Here, I generated a dataset of DNA sequences from one mitochondrial region and seven nuclear DNA fragments to test the hypothesis that eastern and western *A. carneipes* breeding colonies form two independent genetic clusters, as suggested by observed high philopatry and evidence of different foraging distributions and strategies during the breeding and non-breeding season. However, to more rigorously test the assumption of foraging segregation during the non-breeding period, I inspected mtDNA sequences from fisheries' bycatch individuals obtained in Japanese waters in the North Pacific Ocean. In addition, as flesh-footed shearwater colonies are threatened by anthropological-driven changes, such as fisheries bycatch of individuals around their breeding colonies and during their transequatorial migration (Baker and Wise, 2005; Waugh *et al*., 2016), which has led to a decline of ~40% of the world's population (Lavers, 2014; Reid *et al*., 2013a), I tested for historical population size variation to assess whether different foraging populations have experienced and survived similar demographic changes in the past.

Materials and Methods

Sample collection

We collected blood samples from *A. carneipes* individuals (*n*=139) from 12 breeding colonies (Figure 3 – 1, Table 3 – 1). Colonies were pooled into five geographic regions for analysis, with maximum inter-colony distance <150 km within a region and >2000 km between regions: Lord Howe Island (*n*=43), New Zealand (*n*=30), South Australia (*n*=20), Western Australia (*n*=45), Saint-Paul Island (*n*=1). Feathers sampled from a non-breeding

area in the Sea of Japan, North Pacific Ocean (birds caught as fisheries bycatch in Japanese waters, *n*=9), were provided by The Burke Museum. All blood samples from Lord Howe Island, Western Australia, South Australia and Lady Alice Island (New Zealand) were collected from flesh-footed shearwaters under Animal Ethics Permit number AEC 021028/02 issued by the Department of Environment, Climate Change and Water (DEWNR). The National Institute of Water and Atmospheric Research (NIWA) provided samples from the Coromandel Peninsula (New Zealand), and the Paris Museum provided the sample from Saint-Paul Island. Blood was preserved in Queen's lysis buffer (Seutin *et al.*, 1991). Museum Identification numbers are shown in the Supplementary Information SI. 3 – 1.

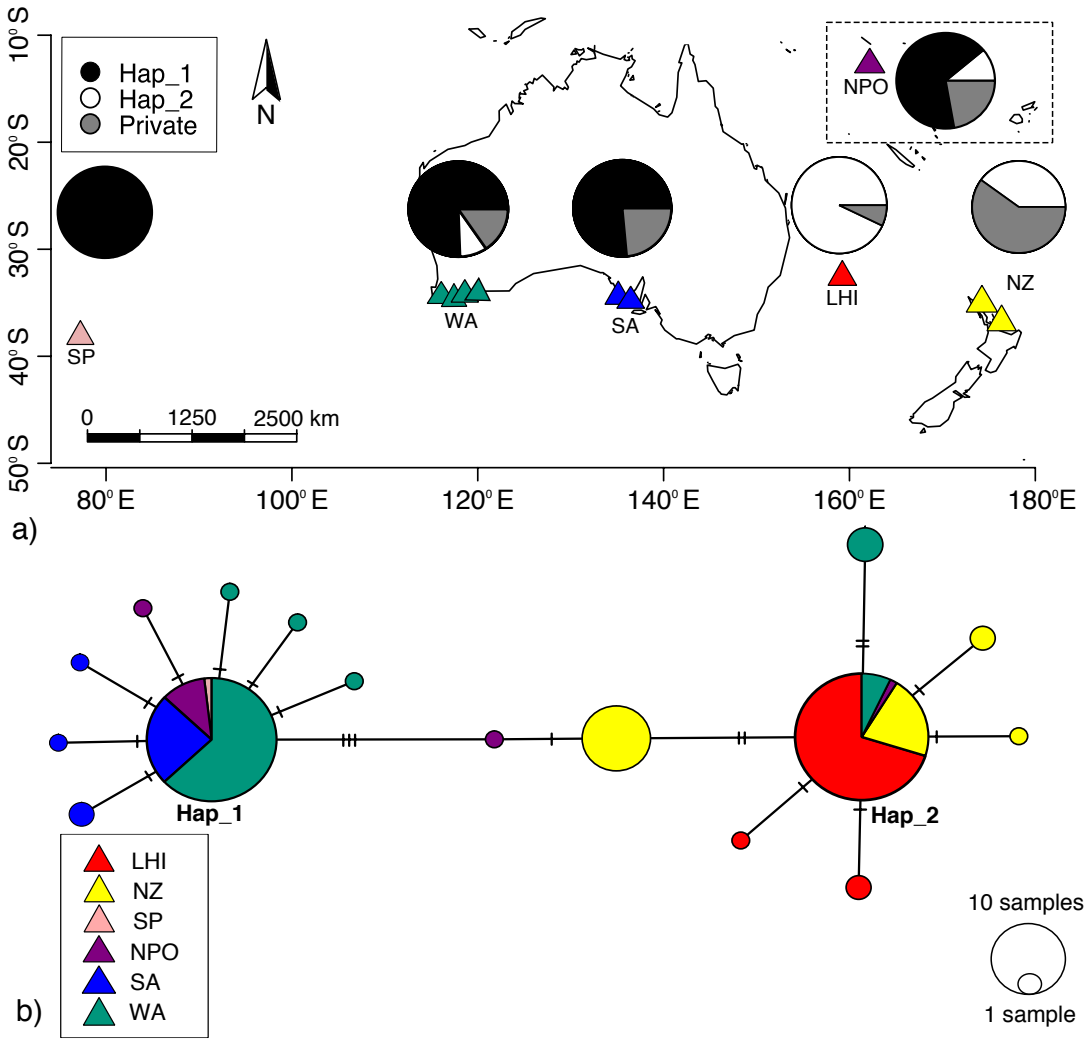

Figure 3 – 1 Sampling locations of flesh-footed shearwaters (*A. carneipes*) and mtDNA haplotype network based on the TCS algorithm. a) Sampling locations of breeding individuals: LHI = Lord Howe Island, NZ = New Zealand, SA = South Australia, WA = Western Australia, SPI = Saint-Paul Island. NPO = fisheries' bycatch from the North Pacific Ocean. Pie charts representing shared vs. private haplotypes of Cytochrome *b* b) Haplotype network of mtDNA sequences based on the TCS algorithm. Haplotypes are represented by circles, where the size of each circle is proportional to the frequency of the corresponding haplotype. Lines on connecting branches represent mutations.

Table 3 – 1 Sampling sites and characterization of genetic diversity in *A. carneipes* for the mitochondrial Cytochrome *b* gene and seven nuclear DNA fragments. Number of birds sampled (*n*), localities and geographic coordinates from breeding colonies of *A. carneipes*. Genetic statistics for each sample as mean haplotypic diversity (H_d), haplotype ratios (X_H), nucleotide diversity (P_i), and nucleotide ratios (π_R).

Locality	Pop. Size	*n*	Coordinates		Haplotypic diversity		Nucleotide diversity	
	(breeding pairs)		Latitude	Longitude	H_d	X_H	P_i	π_R
Lord Howe Island (LHI)	14,800 – 18,800[a]	**43**			0.489	0.441	0.00395	0.740
Ned's Beach	–	34	31°51'S	159°07'E				
Clear Place	–	3	31°52'S	159°08'E				
Middle Beach	–	6	31°52'S	159°07'E				
New Zealand (NZ)	10,000 – 15,000[b]	**30**			0.652	0.444	0.00412	1.201
Lady Alice Island	~1000[c]	15	35°54'S	174°44'E				
Coromandel Peninsula	<1000[c]	15	36°80'S	175°48'E				
South Australia (SA)	800 – 3000[a]	**20**			0.608	0.486	0.00420	0.871
Lewis Island	211 ± 121[d]	13	34°57'S	136°01'E				
Smith Island	1613 ± 924[d]	7	35°00'S	136°01'E				
Western Australia (WA)	18,300 – 35,900 [d]	**45**			0.608	0.656	0.00471	1.023
Shelter Island	827 ± 690[d]	13	35°03'S	117°41'E				
Sandy Island	3439 ± 1917[d]	23	34°51'S	116°02'E				
Breaksea Island	1862 ± 12226[d]	6	35°04'S	118°03'E				
Coffin Island	<200[d]	3	35°00'S	118°12'E				
Saint Paul Island (SP)	~100[e]	**1**	38°84'S	77°83'E	–	–	–	–
North Pacific Ocean (NPO)	–	**9**			0.583[f]	0.250[f]	0.0008[f]	0.264[f]
Total		**148**						

[a]Reid *et al*. (2013), [b]Waugh *et al*. (2013), [c]Taylor (2000), [d]Lavers (2014), [e]Roux (1985), [f]P_i and H_d for Cytochrome *b*

Mitochondrial and nuclear DNA sequencing

Genomic DNA was extracted from 148 individuals using a Qiagen DNeasy® Blood and Tissu kit following the manufacturer's protocol. Extracted DNA was quantified using a NanoDrop 8000 spectrophotometer (Thermo Fisher Scientific, USA). I determined the nucleotide sequences of a 858 bp fragment of the mitochondrial Cytochrome *b* gene for 145 *A. carneipes* individuals using primers L14841 and H15547 (Kocher *et al*., 1989), and 101-132 individuals for ~500 bp fragments of seven nuclear DNA fragments (*4080*, *18503*, *20454*, *22519*, *Pema01*, *Pema07*, *Pema14*) (Backström *et al*., 2008; Patterson *et al*., 2011; Silva *et al*., 2011). Amplification of nuclear DNA from fisheries' bycatch samples was unsuccessful. The exact number of individuals sequenced for each locus from the five regions (Lord Howe Island, New Zealand, South Australia, Western Australia, Saint-Paul Island) and the non-breeding area in the North Pacific Ocean and associated GenBank Accession numbers are shown in the Supplementary Information SI. 3 – 2. Primer sequences, optimal annealing temperatures and approximate locus length for seven nuclear DNA fragments in *A. carneipes* are shown in the Supplementary Information SI. 3 – 3.

All fragments were PCR amplified with MangoTaq™ DNA polymerase following the manufacturer's protocol (Bioline Inc.). PCR reactions were performed in 25 µL using 50–100 ng DNA, and final concentrations of 0.5 U DNA polymerase, 0.2 mM of each dNTP, 1.5 mM $MgCl_2$ and 0.3 µM of each primer. The thermal cycling profiles included an initial denaturation at 95°C for 1 minute followed by 29 cycles of denaturation at 95°C for 30 s, annealing for 40 s, and extension of 72°C for 90 s, with a final extension of 72°C for 10 minutes. Negative controls were included with each set of PCRs.

Nucleotide sequences were determined on both strands of PCR products using a 3730xl DNA Analyzer (Applied Biosystem®) at Macrogen Inc., Korea. Sequences were aligned using the MUSCLE algorithm (Edgar, 2004) in CODONCODE ALIGNER v3.7.1.1 (CodonCode Corporation). For nuclear DNA sequences containing multiple heterozygous positions, I used the Bayesian method implemented in PHASE v2.2.1 (Stephens *et al*., 2001) to reconstruct the haplotype phase of the sequences. I ran the algorithm three times from different starting points to verify convergence with 10,000 iterations per locus, and discarded the first 1,000 samples as burn-in and the output probability threshold was set to 80%. The program SEQPHASE (Flot, 2010) was used during this process.

Quantifying and testing assumptions of genetic variation

Haplotypic diversity *h* (Nei, 1987) and nucleotide diversity π (Tajima, 1983) were calculated for mtDNA and nuclear DNA sequences with SPADS v 1.0 (Dellicour and Mardulyn, 2014). To detect potential hotspots of genetic diversity (e.g. refuge or secondary contact zones), haplotype ratios X_{ii}, and nucleotide diversity ratios π_{ii} (Mardulyn *et al*., 2009) were calculated for each region. To test whether patterns of genetic variation deviated from neutral expectations, Tajima's *D* (Tajima, 1983) and Fu and Li's *D** (Fu and Li, 1993) tests were performed using DNASP v 5.10 (Librado and Rozas, 2009) for each region, and for all individuals grouped as a single population, for each genetic marker.

Population genetic structure

Estimates of population differentiation (F_{st}, G_{st} and N_{st}) among four regions (Lord Howe Island, New Zealand, South Australia and Western Australia) were determined for Cytochrome *b* and seven nuclear DNA fragments using SPADS. Fisheries' bycatch individuals sampled during the non-breeding period in the Sea of Japan were only included in population

differentiation analyses for Cytochrome *b* to assess their genetic connectivity with individuals sampled from the four breeding regions. The statistical significance of indices was assessed by 10,000 random permutations of individuals among geographical regions. TCS haplotype networks (Clement *et al*., 2000) were inferred for mitochondrial and nuclear DNA sequences, and the frequencies of haplotypes depicted using PopART (http://popart.otago.ac.nz).

To define best clustering (*K*) of regions a posteriori based on genetic differentiation (Lord Howe Island, New Zealand, South Australia and Western Australia), AMOVA Φ-statistics (Φ_{SC} Φ_{ST} Φ_{CT}) (Excoffier *et al*., 1992) were calculated on all loci for *K*=2 (seven possible groupings), *K*=3 (six possible groupings) and *K*=4 (see Table 3 – 3 in the section 'Results') with 10,000 permutations of individuals among regions using SPADS. AMOVA Φ-statistics were also calculated for Cytochrome *b* only for *K*=2–4 following the same procedure.

Gene flow and divergence times

As F_{st} cannot distinguish between a situation of high gene flow among colonies that have diverged a long time, from one of a relatively recent shared history but no ongoing gene flow, I used the isolation with migration model (Hey and Nielsen, 2007) to assess the demographic history of *A. carneipes* colonies. Two methods were used for comparison. First, I estimated the time of divergence between eastern (Lord Howe Island and New Zealand) and western (South Australia, Western Australia and Saint Paul Island) regions considering the best genetic clustering as *K*=2 (Figure 3 – 2a). I used IMa and its model of isolation with migration (Hey, 2010) to simultaneously estimate migration (m) and lineage divergence time (t) between these two groups of colonies. Second, I assessed demographic history of *A. carneipes* considering *K*=4, with four regions (Lord Howe Island, New Zealand, South Australia, Western Australia) (Figure 3 – 2b). Here, I used IMa2 (Hey, 2010), that allows

analysis of more than two regions. I defined the topology of the population tree implemented for the four distinct regions, and information on the ordering of the internal nodes in time, based on *F*-statistics and AMOVA Φ-statistics (Φ_{SC} Φ_{ST} Φ_{CT}). Alternate topologies were also tested (Figure 3 – 2b) to investigate potential bias of the results due to incorrect assumption of the topology and the ordering of internal nodes. Only gene flow between sister populations was allowed to reduce the number of parameters and the size of the overall model (Hey, 2010).

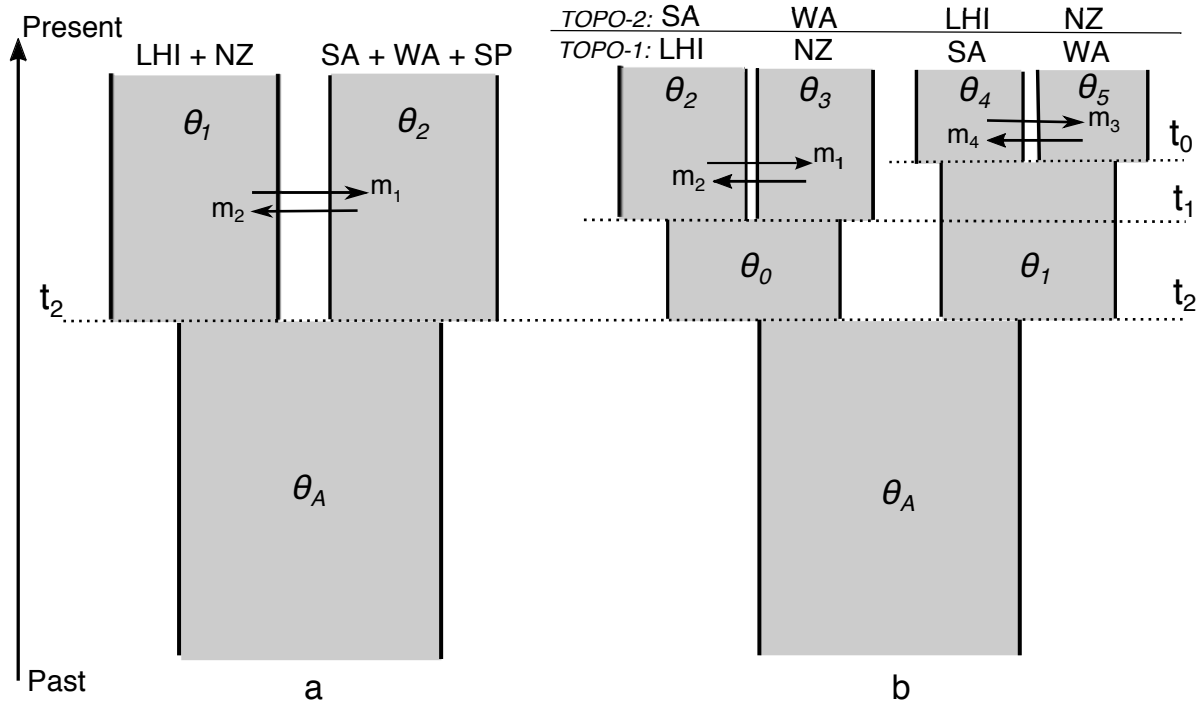

Figure 3 – 2 Hypotheses of demographic history of *A. carneipes* colonies: Lord Howe Island (LHI), New Zealand (NZ), South Australia (SA), Western Australia (WA) and Saint Paul Island (SPI) as implemented in IMa and IMa2. a) Hypothesis implemented in IMa. b) Hypothesis implemented in IMa2. θ = population size, m = migration rate and t = divergence time.

The isolation with migration model is based on several assumptions including neutrality, random mating in ancestral and descendent populations, and free recombination between but not within loci (Nielsen and Wakeley, 2001; Hey and Nielsen, 2004). Lack of recombination within nuclear DNA fragments was tested using the four-gamete test as described by Hudson and Kaplan, (1985). Three loci suspected to have experienced recombination (*4080, 18503, 20454*) were discarded. Mutation rates were given as priors to the analysis with $\mu = 1.89 \times 10^{-8}$ and 3.6×10^{-9} substitution/site/year for Cytochrome *b* and nuclear DNA fragments respectively, as recommended for other seabirds (Axelsson *et al*., 2004; Weir and Schluter, 2008). To assess the estimates of demographic parameters, I assumed a generation time T = 18.3 years (BirdLife International http://datazone.birdlife.org/). I implemented the Hasegawa-Kishino-Yano (HKY) (Hasegawa *et al*., 1985) model for the mitochondrial data, and the infinite sites mutation model (IS) (Kimura, 1969) for the nuclear DNA fragments. IMa/IMa2 exploratory runs were performed to assess a range of prior distributions that include most of the range over which the posterior density is not trivial. Analyses were then run three times with different seed numbers to test for convergence, with 200,000,000 sampled steps following a discarded burn-in of 20,000,000 steps, with a two-step linear heating scheme with five chains. Parameter trend line plots and values of effective sample sizes (ESS) were inspected after each run, and results were discarded based on a selection criterion ESS<200 to assure accurate estimates of posterior distributions.

Effective population size change analyses

A Bayesian coalescent MCMC model was used to estimate historical demographic fluctuations of *A. carneipes* colonies, grouped in four regions, over time using Cytochrome *b* and seven nuclear DNA fragments as implemented in BEAST2 v.2.4.4. (Bouckaert *et al*., 2014). For our demographic model, I applied the Coalescent Extended Bayesian Skyline Plot

(EBSP). This model is based on the generalized skyline plot, which, assuming a single panmictic population, estimates fluxes in population size (*N*) through time but allows the analysis of multiple loci (Drummond *et al*., 2005). As violations of panmixia can lead to false signals of population decrease under EBSPs (Heller *et al*., 2013), I performed analyses of regions separately. The nucleotide substitution model that best fit the data was selected using the lowest Bayesian Information Criteria (BIC) in jMODELTEST v2.1.10 (Guindon and Gascuel, 2003) for each genetic marker as recommended by Posada and Buckley (2004). Three BEAST runs were conducted for each geographic region under a strict molecular clock (n_{TOT} runs = 12) with substitution rates as above. Additional runs were performed after having discarded the three nuclear loci suspected to have experienced recombination (*4080, 18503, 20454*) under the same parameters (n_{TOT} runs = 12). The scale factor for the population size was set at 0.5 for Cytochrome *b* and 2 for nuclear DNA fragments, reflecting their different inheritance and ploïdy. MCMC chains were run for 200,000,000 iterations, sampling the posterior distribution every 20,000 iterations with the first 10% discarded as burn-in. The XML file for each set of analyses generated with BEAUti v.2.4.4 are available as Supplementary Material SM (available online). Parameter trend line plots and values of effective sample sizes (ESS) were inspected after each run and results were discarded based on ESS<200 using TRACER v1.6 (Rambaut *et al*., 2015) to assure accurate estimates of posterior distributions. MCMC analyses for the same dataset (groups of population/loci) were combined with LogCombiner v.2.4.4. To characterise the magnitude of *Ne* change in each lineage, I fit median *Ne* and time, obtained from the Bayesian skyline posterior distribution, to a linear model using a modified version of the plotEBSPR script (http://beast2.org/tutorials/) implemented in R v.3.1.2.

Results

I sequenced 858 bp of the mtDNA Cytochrome *b* gene, and a total of 3328 bp comprising seven nuclear DNA fragments in up to 148 *A. carneipes* individuals from five breeding regions (Lord Howe Island, New Zealand, South Australia, Western Australia and Saint-Paul Island), and one non-breeding area (Sea of Japan in the North Pacific Ocean). No length mutations were observed. A total of 16 (Cytochrome *b*), 24 (*4080*), 21 (*18503*), 40 (*20454*), 7 (*22519*), 5 (*Pema01*), 10 (*Pema07*) and 14 (*Pema14*) haplotypes were defined. Variable sites in the mtDNA marker Cytochrome *b*, shared (Hap_1 and Hap_2) vs. private (Hap_A–N) haplotypes, nucleotide and codon positions of variable sites, and substitution type (all are transitions), are shown in Supplementary Information SI. 3 – 4. Global haplotypic diversity (H_d) and nucleotide diversity (P_i) for each region are reported in Table 3 – 1. H_d and P_i for each genetic marker and each colony are reported in Supplementary Information SI. 3 – 5. No significant difference in global nucleotide diversities (π) among regions was detected (One-way ANOVA; H_0 = means of π are equal in all regions; $F_{1,4} = 0.033$; *p*-value = 0.992; see π values combined over all loci in Table 3 – 1). Tajima's *D* showed significant positive values in one nuclear locus (*20454*) for all regions and when all individuals were grouped as a single population (Supplementary Information, SI. 3 – 6). Fu and Li's *D** tests showed positive values for the same nuclear locus (*20454*) in New Zealand and South Australia (Supplementary Information SI. 3 – 6).

Population genetic structure

Haplotype networks (mtDNA, Figure 3 – 1b; nuclear DNA fragments, Supplementary Information SI. 3 – 7), mtDNA haplotype frequencies (Figure 3 – 1a) and significant *F*-statistic values over eight loci (global $F_{st} = 0.202$, $p<0.005$; global $G_{st} = 0.118$, $p<0.005$) for

mitochondrial and nuclear DNA sequences support strong structure of genetic variation among regions (global F_{st} for mitochondrial DNA sequences only = 0.657, $p<0.005$; global F_{st} for nuclear DNA sequences only = 0.053, $p<0.005$). A significant phylogeographic signal (global N_{st} = 0.132, $p<0.005$) supports these results. The F_{st} pairwise matrix indicated a greater genetic structure between the eastern (Lord Howe Island and New Zealand) and the western (South Australia, Western Australia and Saint-Paul Island) parts of the breeding distribution, but still with significant difference between New Zealand and Lord Howe Island (Table 3 – 2). The magnitude of population genetic differentiation at the mtDNA marker was in all cases higher than at nuclear DNA. No significant genetic structure was observed between Western Australia and South Australia. For Cytochrome *b*, significant F_{st} was observed among fisheries' bycatch individuals from the North Pacific Ocean and eastern regions (Lord Howe Island, New Zealand), but not from western regions (South and Western Australia). The common haplotype from western regions was represented in the single Saint-Paul Island individual, but further samples are required to assess whether the Saint Paul Island colony is distinct from other western regions. Two private haplotypes were observed in the fisheries' bycatch samples.

Table 3 – 2 Pairwise differentiation (F_{st}) matrix among *A. carneipes* samples, including fisheries' bycatch individuals from the North Pacific Ocean. Below diagonal: pairwise F_{st} for Cytochrome *b* and seven nuclear DNA fragments. Above diagonal: Pairwise F_{st} matrix for Cytochrome *b*.

	LHI	**NZ**	**SA**	**WA**	**NPO**
Lord Howe Island (LHI)	–	0.457*	0.964*	0.768*	0.904*
New Zealand (NZ)	0.139*	–	0.824*	0.609*	0.667*
South Australia (SA)	0.333*	0.253*	–	0.080	0.119
Western Australia (WA)	0.238*	0.190*	0.019	–	-0.085
North Pacific Ocean (NPO)	–	–	–	–	–

* *p*-values < 0.00833 after sequential Bonferroni correction

AMOVA Φ-statistics on combined genetic markers showed that greatest spatial structuring of genetic variation for clustering *K*=2 was eastern and western regions (Group 1=Lord Howe Island, New Zealand; Group 2=South Australia, Western Australia, Saint-Paul Island, Table 3 – 3) where 17.7% of variance was explained by the region grouping. For *K*=3, the greatest spatial structuring was as above but Lord Howe Island and New Zealand separated (Group 1=Lord Howe Island, Group 2=New Zealand, Group 3=South Australia, Western Australia, Saint-Paul Island, Table 3 – 3), where 20.2% of variance was explained by the region grouping. AMOVA Φ-statistics for Cytochrome *b* showed the same greatest spatial structures as above for *K*=2 where 71.4% of variance was explained by the region grouping, and *K*=3 where 70% of variance was explained by the region grouping (Supplementary Information SI. 3 – 8).

Gene flow and divergence times

Implementations of the isolation-with-migration models (IMa and IMa2) using nuclear DNA fragments and mitochondrial DNA resulted in unimodal posterior density curves of migration parameters, which were convergent across runs. The time of divergence between eastern and western colonies as implemented in IMa was ~28,000 years ago (11,700 – 100,000, 90% HPD), and very low gene flow was inferred (~0) (Figure 3 – 3; Supplementary Information SI. 3 – 9). Time of divergence between eastern and western colonies as implemented in IMa2 showed similar results to the one obtained with IMa (~28,000 years; 9,800 – 76,000, 90% HPD). The time of divergence between Lord Howe Island and New Zealand, and between Western and South Australia under Topology 1 and Topology 2 (Figure 3 – 2) were convergent, ~3,000 years (2,000 – 7,000, 90% HPD) and ~2,100 years (800 – 6,000, 90% HPD) respectively (Figure 3 – 4; Supplementary Information SI. 3 –10). Gene flow was

higher between western than eastern colonies, but was still very low (Figure 3 – 4; Supplementary Information SI. 3 – 10).

Table 3 – 3 AMOVA Φ-statistics (Φ_{sc} Φ_{st} Φ_{ct}) (Excoffier *et al*., 1992) for *A. carneipes* breeding regions. A total of seven (*K*=2), six (*K*=3) and one (*K*=4) groupings of breeding regions were tested. *p*-values for Φ_{sc} are based on permutations of sampled sequences across regions within the same group, *p*-values for Φ_{st} are calculated based on permutations of sampled sequences among regions without regard to their original group, and *p*-values for Φ_{ct} are based on permutations of whole regions among groups.

Groups	Φ_{sc}	Φ_{st}	Φ_{ct}
***K*=2**			
[LHI, NZ][SA, WA]	0.083*	0.246*	**0.177***
[LHI, SA][NZ, WA]	0.251*	0.177*	-0.099
[LHI, WA][SA, NZ]	0.244*	0.173*	-0.094
[LHI] [NZ, SA, WA]	0.169*	0.221*	0.063*
[NZ] [LHI, SA, WA]	0.218*	0.182*	-0.046
[SA] [LHI, NZ, WA]	0.203*	0.205*	0.003
[WA] [LHI, NZ, SA]	0.210*	0.195*	-0.019
***K*=3**			
[LHI][NZ][SA, WA]	0.024*	0.221*	**0.202***
[LHI][SA][NZ, WA]	0.204*	0.200*	-0.005
[LHI][WA][NZ, SA]	0.249*	0.197*	-0.069
[NZ][SA][LHI, WA]	0.240*	0.183*	-0.075
[NZ][WA][LHI, SA]	0.310*	0.187*	-0.178
[SA][WA][LHI, NZ]	0.126*	0.219*	0.106
***K*=4**			
[LHI][NZ][SA][WA]	-	**0.202***	-

*$p<0.05$

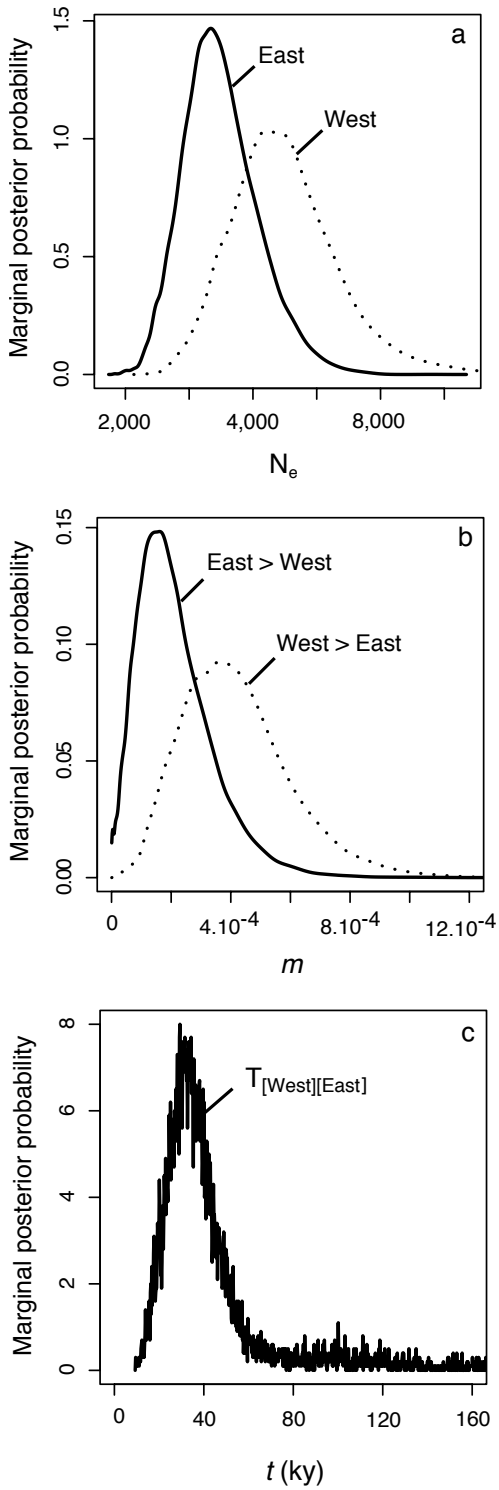

Figure 3 – 3 Marginal posterior distribution of the parameters for the Isolation with Migration model estimated for eastern and western *A. carneipes* breeding regions (*K* = 2). a) population sizes (N_e). b) migration (*m*). c) time of divergence (t_2, years) between eastern and western colonies.

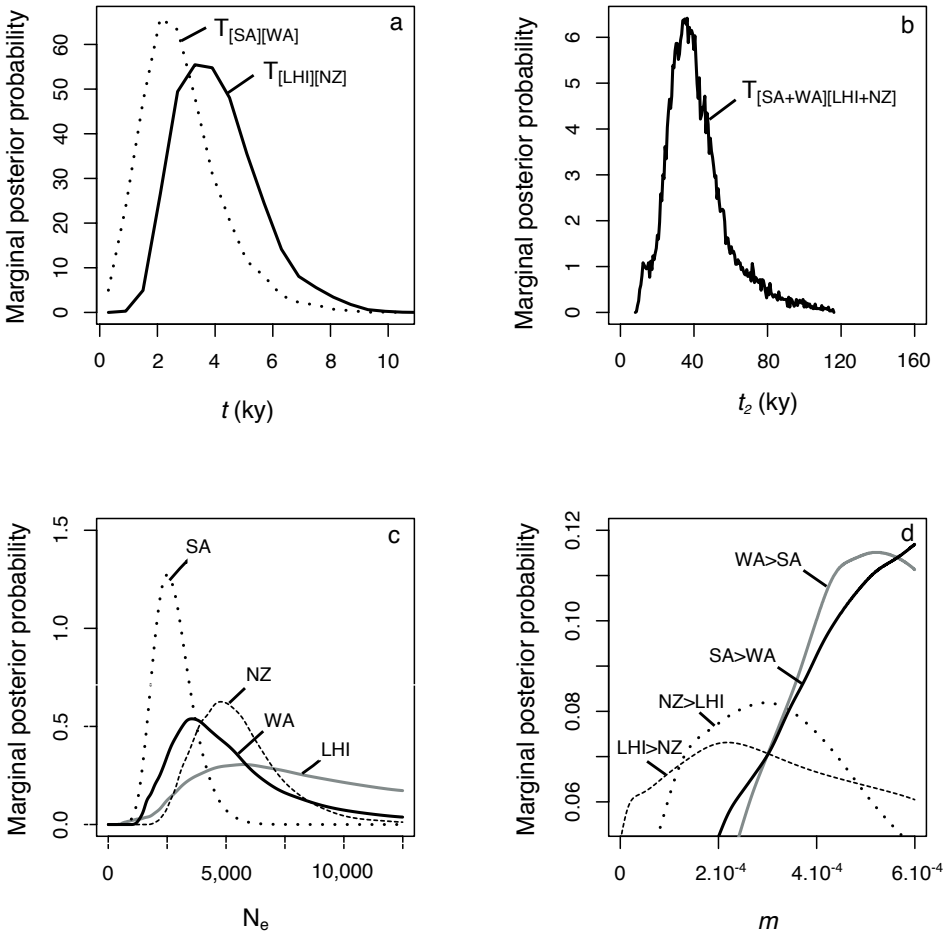

Figure 3 – 4 Marginal posterior distribution of the parameters for the Isolation with Migration model estimated for *A. carneipes* breeding regions (*K* = 4). a) time of divergence between *A. carneipes* colonies (*t*, years). LHI = Lord Howe Island, NZ = New Zealand, WA = Western Australia, and SA = South Australia. b) time of divergence between eastern and western colonies (t_2, years). c) population sizes (N_e). d) migration (*m*).

Effective population size change analyses

All models tested with the Extended Bayesian Skyline Plot (EBSP) showed similar results, although models integrating loci suspected to be undergoing recombination (*4080, 18503, 20454*) showed broader 95% HPD for Lord Howe Island (Figure 3 – 5; Supplementary Information SI. 3 – 11). My demographic reconstruction indicates two distinct phases for the

four regions, including one phase of stability followed by one phase of population expansion with the last 5kyr (Figure 3 – 5).

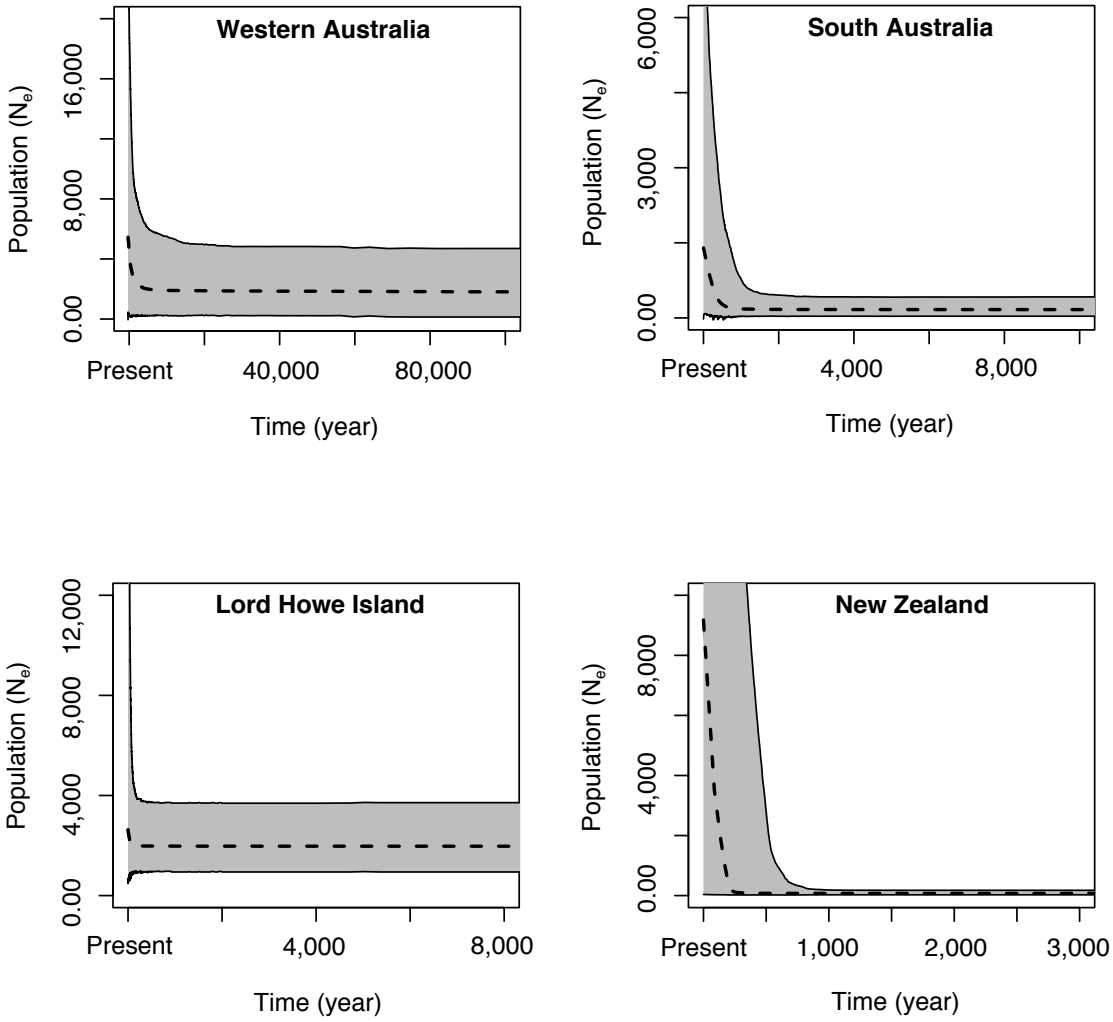

Figure 3 – 5 Extended Bayesian Skyline Plot (EBSP) showing demographic reconstruction of *A. carneipes* effective population size (N_e) through time for Cytochrome *b* and seven nuclear DNA fragments. Dotted curves and solid curves represent the median Bayesian Skyline reconstruction and 95% HPD intervals, respectively.

Discussion

Genetic differentiation among A. carneipes colonies

Haplotype networks, AMOVA *F*-statistics, and Isolation with Migration models for mitochondrial and nuclear DNA sequences, indicated low gene flow and long divergence between *A. carneipes* colonies breeding east of Australia (Lord Howe Island and New Zealand) and western breeding colonies (Western Australia, South Australia, and Saint-Paul Island). The divergence between these regions (~28,000 years) roughly corresponds to the beginning of the Last Glacial Maximum (LGM). Previous studies showed that in seabirds, availability of new breeding habitat due to sea level change and the latitudinal migration of oceanic fronts influenced their global distribution and phylogeographic structure (Techow *et al*., 2010). Since the flesh-footed shearwater populations diverged, gene flow between eastern and western colonies has possibly been restricted by unsuitable breeding areas throughout the southeastern coast of Australia (Peck and Congdon, 2004; Byrne, 2008), as well as sea level changes leading to the relocation of breeding colonies during periods of warming and exposure of Bass Strait (Dartnall, 1974; Lambeck *et al*., 2002). Northward range shifts may also have increased the isolation of eastern and western populations, as has been hypothesized for other temperate marine Australian taxa (Burridge, 2000; Fraser *et al*., 2009). In addition, the star-like phylogeny of both the western and eastern regions may reflect spatially distinct refugia, which is consistent with areas of climatic suitability from species distribution modeling with projected LGM climatic conditions (Buckley *et al*., 2010; Nistelberger *et al*., 2014), followed by a range expansion. However, old divergences and an apparent lack of contemporary gene flow between eastern and western regions provide evidence of signatures of both historical and contemporary processes affecting the genetic structure of *A. carneipes* colonies.

Overlap in non-breeding distributions between colonies

Despite suggestions from telemetry of distinct routes of migration and distribution of individuals from western and eastern colonies during the non-breeding season, individuals sampled during the non-breeding period in the Sea of Japan were assigned to eastern and western colonies. A majority of fisheries' bycatch specimens (66%) possessed Haplotype_1, which has only otherwise been observed from Western and South Australia, where it is common (75% of individuals). As this haplotype was not observed among 73 individuals sampled from Lord Howe Island and New Zealand, if it exists there, its frequency is less than 1.5%. Therefore, it is unlikely that the presence of Haplotype_1 in a high proportion of bycatch individuals from the Sea of Japan can be explained in the absence of migration by Western or South Australian individuals. The only possible alternate explanation is that western birds founded a new and presently unsampled and unknown colony in the eastern part of the species range, and these birds have adopted the migration route of Lord Howe and New Zealand individuals, and somehow constituted a large proportion of our bycatch sample, despite their source colony being undocumented. However, each of these required events (establishment of new colony in the east by western individuals, presently unknown colony, adoption of new migration route, and majority composition of the bycatch samples despite their source colony being unknown to science) seems unlikely, and in combination, discount the possibility that Western or South Australian birds are not migrating to the Sea of Japan in the North Pacific Ocean. Furthermore, the stable isotope and trace element analysis conducted by Lavers (2013) also suggested that some Sea of Japan bycatch individuals were derived from Western and South Australian breeding colonies. Based on our observations, previous studies may have falsely invoked distinct non-breeding distributions for population genetic structure in seabirds in instances where there are limited data supporting distinction

of non-breeding distributions. As a result, genetic structure among flesh-footed shearwater colonies can hardly be explained by distinct non-breeding distributions.

Foraging distinction between eastern and western colonies during the breeding season

Significant genetic differentiation inferred between Lord Howe Island and New Zealand *A. carneipes* breeding colonies, as previously observed in a study using microsatellite markers (Hardesty *et al*., 2013), supports differences in foraging strategy during the breeding season as a factor influencing dispersal between *A. carneipes* colonies, rather than geographic distances or differences in migration routes. The flesh-footed shearwater is a central place forager restricted within a certain range of their breeding site (Ashmole, 1971), and has been shown to return to similar foraging areas during the breeding season from one year to the next (Kinsky, 1957; Reid, 2011). Individuals breeding on Lord Howe Island forage off the east coast of Australia not further than 1000 km from their breeding sites (Reid *et al*., 2012; Thalmann *et al*. 2009), as expected in migratory central-place foragers (Orians and Pearson, 1979). This preference for foraging areas likely reflects western boundary currents moving south along the east coast of Australia that drive strong oceanographic features such as up-wellings in the Tasman Sea, increasing prey availability in this area (Ridgway and Dunn, 2003). Conversely, *A. carneipes* individuals breeding on New Zealand islands mostly forage over continental shelves north of the sub-tropical convergence (Rayner *et al*., 2011).

Isotopic ratio analysis and shipboard observations suggest that individuals from eastern and western regions have distinct foraging strategies. Individuals breeding east of Australia may forage in more inshore waters (<1000 km, Reid *et al*., 2012) and at a higher trophic level than individuals breeding at western colonies, believed to forage in offshore waters (Bond and Lavers, 2014; Lombal unpublished data). This may be explained by El Niño–Southern

Oscillations (ENSO) events affecting the Cape Leeuwin Current near the western coast of Australia and lower associated prey availability, and the increase of industrial fishing in this region (Lindsey, 1986; Taylor, 2000; Cheung *et al*., 2012; Bond and Lavers, 2014). These observations are compatible with distinct foraging distributions during the breeding season affecting gene flow among flesh-footed shearwater colonies, which is consistent with Friesen (2015) showing that 91% of seabird species that either feed inshore or have population-specific foraging areas show some evidence of restriction in gene flow among colonies.

Intraspecific morphological variation and taxonomic status

Our observations of spatial genetic variation are consistent with previous morphological differences observed between flesh-footed shearwaters. Hindwood (1945) recognized two subspecies: *Puffinus carneipes carneipes* at the Recherche Archipelago and other islands of south-west Western Australia, and *Puffinus carneipes hullianus* for Lord Howe Island and New Zealand, differing from the nominate subspecies by a more robust bill and longer wing. While flesh-footed shearwaters from eastern and western colonies do not show reciprocal monophyly for mtDNA alleles, this could be explained by the rapid evolution of phenotypic variation compared to sorting of MtDNA variation in abundant taxa, which can take tens of thousands of years to evolve (Avise, 2000; Mayr, 1970). In addition, as both petrels and albatrosses show unusually low levels of genetic divergence (Nunn and Stanley, 1998), the power of genetic analysis to resolve taxonomic uncertainties is usually reduced in Procellariiformes (Burg and Croxall, 2001; Abbott and Double, 2003).

It is often difficult to determine whether populations have diverged to the extent that they should be considered as distinct species (Harrison, 1998; Sites and Marshall, 2004). Speciation is thought to be a gradual process in animals, with complete reproductive isolation developing at the final stage (Mayr, 1963). As a result, the Biological Species Concept (BSC;

Mayr, 1942) defines species as reproductively isolated groups of populations, which can only be directly observed if populations coexist in space and time, and therefore does not apply to allopatric populations (Mayr and Short, 1970; McKitrick and Zink, 1988; Helbig *et al*., 2002). Conversely, the Phylogenetic Species Concept (PSC; Cracraft, 1983) argues that reproductive isolation should not be a part of species concepts, and instead requires that a) species be monophyletic groups, and b) species be distinguishable from other such groups in one or more characters (e.g. diagnosability; Helbig et al., 2002) The recognition of Evolutionary Significant Units (ESU; Avise, 1989) also requests reciprocal monophyly for mtDNA alleles but only significant divergence of allele frequencies at nuclear loci, as this concept considers phylogenetically unsorted alleles at nuclear loci. These requirements are comparable to the Agreement on the Conservation of Albatrosses and Petrels (ACAP) guidelines adapted from Helbig (2002) which has adapted the concept of monophyly by the condition that taxa are 'likely to retain their genetic and phenotypic integrity in the future' (ACAP Document 11 of AC2).

Moritz (1994) stressed that populations that do not show reciprocal monophyly for mtDNA alleles, yet have diverged in allele frequencies, are significant for conservation in that they represent populations connected by such low levels of gene flow that they are functionally independent. Therefore, Management Units (MUs) are recognized as populations with significant divergence of allele frequencies at nuclear or mitochondrial loci, regardless of the phylogenetic distinctiveness of the alleles; MUs address current population structure, allele frequencies and short-term management issues (Moritz, 1994). Eastern and western colonies of flesh-footed shearwaters may not represent cryptic species under the PCS given their lack of phylogeographic structure but evidence of morphometric differences and strong divergence in allele frequencies among colonies give strong support that they are functioning as separate entities and that they should be considered as independent MUs. The flesh-footed

shearwater likely represents a case of incipient speciation, and for which taxonomic decision remains difficult.

Detection of demographic changes

Overall, the similarity of coalescent histories among colonies indicates that, although genetically independent, they have homologous demographic histories, including one phase of stability followed by one recent period of population expansion, occurring in the last 5 kyr. This may be explained by population expansion associated with the LGM, as observed in many high latitude seabird populations (Congdon *et al*., 2000; Hewitt, 2000), although the timing of expansion is much more recent in this case. Furthermore, while coalescent theory is a powerful tool to extract historical demographic information from DNA sequences (Hudson, 1990), Grant (2015) observed that small and very recent population expansions often appear in Bayesian Skyline Plots of simulated populations that did not experience a sudden recent change in size. In fact, this pattern frequently reflects random sampling of the MCMC haplotype trees (Grant, 2015). The flat portion of the BSP, usually interpreted as population stability, is also often misleading as population contractions can promote extinctions of haplotype lineages leading to the loss of information about earlier population history (Grant, 2015). In addition, as slightly deleterious mutations are slowly eliminated by selection, preventing low-frequency mutations from moving to higher frequencies (Charlesworth *et al*., 1993), haplotype frequency distributions shaped by selection are difficult to distinguish from distributions produced by a population expansion. On the other hand, even if I performed analyses of regions separately, violations of panmixia may also have led to false signals of population decrease under EBSPs (Heller *et al*., 2013) given that populations were pooled in regions. For these reasons, and because of the lack of additional significant evidence of demographic changes in flesh-footed shearwater colonies, my BSPs results need to be

interpreted cautiously, and may not represent evidence for novelty of the presently observed population declines.

Consequences for conservation status of flesh-footed shearwaters

In this study, I show a lack of gene flow between *A. carneipes* colonies from Lord Howe Island, New Zealand and localities to the west, indicating that populations have clearly experienced independent evolution for a long time, which may greatly affect long-term viability and persistence of the species within these regions owing to local adaptation and demography independence. The flesh-footed shearwater is now listed as Near Threatened in Birdlife International (2017) IUCN Red list for birds (http//www.birdlife.org), and therefore there is an urgent need to develop a suite of mitigation measures that would reduce the level of bycatch currently being experienced in each of these regions.

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Supplementary Information SI. 3

Genetic divergence between colonies of flesh-footed Shearwater *Ardenna carneipes* exhibiting different foraging strategies

Anicee J. Lombal[1,], Theodore J. Wenner, Jennifer Lavers, Jeremy J. Austin, Eric Woehler, Ian Hutton and Christopher P. Burridge.

[1]Discipline of Biological Sciences, University of Tasmania, Hobart, TAS 7001, Australia.

[2]Anicee.Lombal@utas.edu.au

The following sections are included in this Supplementary Information SI. 3:

SI 3 – 1 Identification numbers of *A. carneipes* samples. The National Institute of Water and Atmospheric Research (Coromandel Peninsula, New Zealand), The Burke Museum (fisheries' bycatch samples from the North Pacific Ocean) and Paris Museum (Saint-Paul Island sample).

Providers	Location	ID #
NIWA* Museum	Coromandel Peninsula (NZ)	90016 80190 90131 90213 90089 90125 70004 70006 70008 80189 70183 80005 70003 80191 -
The Burke Museum	Sea of Japan, North Pacific Ocean	55504 55562 82812 85471 55496 61940 55559 55795 55503
Paris Museum	Saint-Paul Island	58251736

*The National Institute of Water and Atmospheric Research

SI. 3 – 2 Number of *A. carneipes* individuals sequenced for Cytochrome *b* and seven nuclear DNA fragments from five regions and one non-breeding area in the North Pacific Ocean, number of alleles (N_a) and GenBank Accession numbers. *n* = the number of *A. carneipes* individuals sampled for each region, N_a = number of distinct alleles. GenBank accession numbers for each locus and for each genetic marker are provided.

	Lord Howe Island		New Zealand		South Australia		Western Australia		Saint-Paul Island		North Pacific Ocean		GenBank Accession #
	n=43	N_a	*n*=30	N_a	*n*=20	N_a	*n*=45	N_a	*n*=1	N_a	*n*=9	N_a	
Cytochrome *b*	43	3	30	4	17	4	45	6	1	1	9	4	KY443814 – KY443957
4080	38	13	16	7	15	11	43	19	–	–	–	–	KY442874 – KY443097
18503	42	10	6	7	16	11	39	18	–	–	–	–	KY443098 – KY443303
20454	43	12	27	13	19	11	42	20	1	2*	–	–	KY443304 – KY443569
22519	41	3	18	4	20	5	43	5	–	–	–	–	KY443570 – KY443813
Pema01	36	4	14	4	18	5	33	4	–	–	–	–	KY443958 – KY444159
Pema07	41	6	18	5	20	5	41	7	–	–	–	–	KY444160 – KY444399
Pema14	41	4	18	8	17	4	45	8	1	1	–	–	KY444400 – KY444643

*heterozygotes

SI. 3 – 3 Description of primers for seven nuclear DNA fragments tested in *A. carneipes*, fragment sizes (bp) and PCR annealing temperatures (A_T).

Locus	Source	Forward primer sequence	Reverse primer sequence	bp	A_T (°C)
Pema01	(Silva *et al*., 2011)	5'-ACACAGCCCTCCTTCAGAGA-3'	5'-TTAAGGCTGGACGATGCTCT-3'	396	58
Pema07	(Silva *et al*., 2011)	5'-TGCCTCCAGTTTGCTAAGGT-3'	5'-AAAAGGAATTGCAGGTGTGG-3'	508	55
Pema14	(Silva *et al*., 2011)	5'-CCTAATCTTCCCTTTCACATGG-3'	5'-AGCAGTTAAGGGGTGCTGAA-3'	537	55
4080	(Backström *et al*., 2008)	5'-ATGCAGGAGGAGAACATCAC -3'	5'-CTCCAGGATGTATTTGGGAG-3'	352	52
20454	(Backström *et al*., 2008)	5'-GTCCTGTGCCTTGTGTATGA-3'	5'-CATCTCACAGTATTCCAGGC-3'	374	55
22519	(Backström *et al*., 2008)	5'-TTTGAGACATATGAGCAGGC-3'	5'-TGTTTCTGAAGCTTCAAGTC-3'	654	55
18503	(Backström *et al*., 2008)	5'-ATCATTCGAGGACAGTATGG-3'	5'-GCTATTTAATGCAGAGTTTC-3'	503	56

SI. 3 – 4 Variable sites in Cytochrome *b* for *A. carneipes* and their originating colonies. Five regions: Lord Howe Island (LHI), New Zealand (NZ), Western Australia (WA), South Australia (SA), Saint-Paul Island (SP) and fisheries' bycatch samples from the Sea of Japan in the North Pacific Ocean (NPO). *n,* total number of individuals sharing haplotypes. All substitutions are transitions. Nucleotide position is related to sequences submitted to GenBank (see SI 2), with codon position indicated above the sequence.

Haplotype	Nucleotide position																			Total *n*	Frequency of Haplotype per region
	0	0	1	1	3	3	3	4	4	4	5	5	5	6	7	7	7	8	8		
	0	5	3	8	4	6	9	3	4	7	2	2	8	5	0	3	5	1	2		
	2	4	2	3	3	3	1	2	7	7	5	8	8	1	5	5	1	6	3		
	Codon position																				
	2	3	3	3	1	3	1	3	3	3	3	3	3	3	3	3	1	3	1		
Hap_2	G	C	T	A	G	G	G	T	T	T	C	C	C	T	T	A	G	C	T	57	LHI (40), NZ (12), WA (4), NPO (1)
Hap_A	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	LHI (1)
Hap_B	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	2	LHI (2)
Hap_C	A	T	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	1	NPO (1)
Hap_1	A	T	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	54	WA (34), SA (13), NPO (6), SP (1)
Hap_D	A	T	.	.	.	.	.	C	.	C	.	.	T	.	C	.	.	.	C	1	NPO (1)
Hap_E	A	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	15	NZ (15)
Hap_F	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	2	NZ (2)
Hap_G	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	1	NZ (1)
Hap_H	A	T	.	.	.	.	A	C	.	.	.	.	T	.	C	.	.	.	C	1	SA (1)
Hap_I	A	T	.	.	.	.	.	C	.	.	.	.	T	.	C	.	.	T	C	2	SA (1)
Hap_J	A	T	.	.	A	.	.	C	.	.	.	.	T	.	C	.	.	.	C	1	SA (1)
Hap_K	A	T	.	.	.	.	.	C	.	.	.	T	T	.	C	.	.	.	C	1	WA (1)
Hap_L	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	G	.	.	.	4	WA (4)
Hap_M	A	T	.	.	.	.	.	C	C	.	.	.	T	.	C	.	.	.	C	1	WA (1)
Hap_N	A	T	.	G	.	.	.	C	.	.	.	.	T	.	C	.	.	.	C	1	WA (1)

SI. 3 – 5 Characterization of genetic diversity in *A. carneipes* for Cytochrome *b* and seven nuclear DNA fragments. Number of birds sampled (*n*), haplotypic diversity H_d and nucleotide diversity P_i. LHI= Lord Howe Island, NZ= New Zealand, SA= South Australia, WA= Western Australia, SP= Saint-Paul Island, JW= Japanese waters in the North Pacific Ocean.

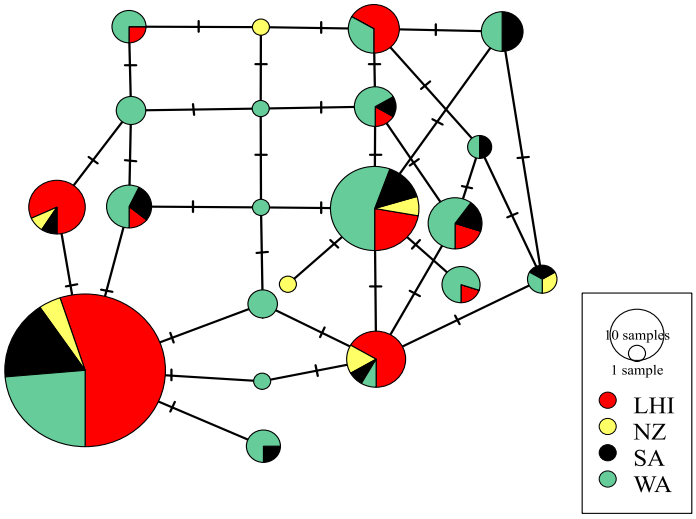
	n	Haplotypic diversity H_d								Nucleotide diversity P_i							
		Cyt b	*4080*	*18503*	*20454*	*22519*	*Pema01*	*Pema07*	*Pema14*	*Cyt b*	*4080*	*18503*	*20454*	*22519*	*Pema01*	*Pema07*	*Pema14*
LHI	43	0.135	0.845	0.635	0.740	0.453	0.525	0.464	0.118	0.00016	0.00776	0.00330	0.01570	0.00136	0.00194	0.00103	0.00035
NZ	30	0.605	0.766	0.878	0.780	0.485	0.373	0.619	0.710	0.00144	0.00580	0.00460	0.01400	0.00131	0.00113	0.00221	0.00250
SA	20	0.420	0.883	0.766	0.748	0.523	0.474	0.663	0.394	0.00143	0.00686	0.00400	0.01650	0.00131	0.00169	0.00195	0.00078
WA	45	0.421	0.884	0.878	0.748	0.517	0.544	0.505	0.372	0.00264	0.00756	0.00455	0.00267	0.001126	0.00163	0.00173	0.00099
SP	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
NPO	9	0.583	–	–	–	–	–	–	–	0.00240	–	–	–	–	–	–	–

SI. 3 – 6 Deviation from neutral expectations: Tajima's *D* test (Tajima, 1983) and Fu and Li's *D test (Fu and Li, 1993) of Cytochrome *b* and seven nuclear DNA fragments for *A. carneipes* individuals.** Five regions: Lord Howe Island, New Zealand, South Australia, Western Australia and Saint-Paul Island and fisheries' bycatch samples from the Sea of Japan in the North Pacific Ocean.

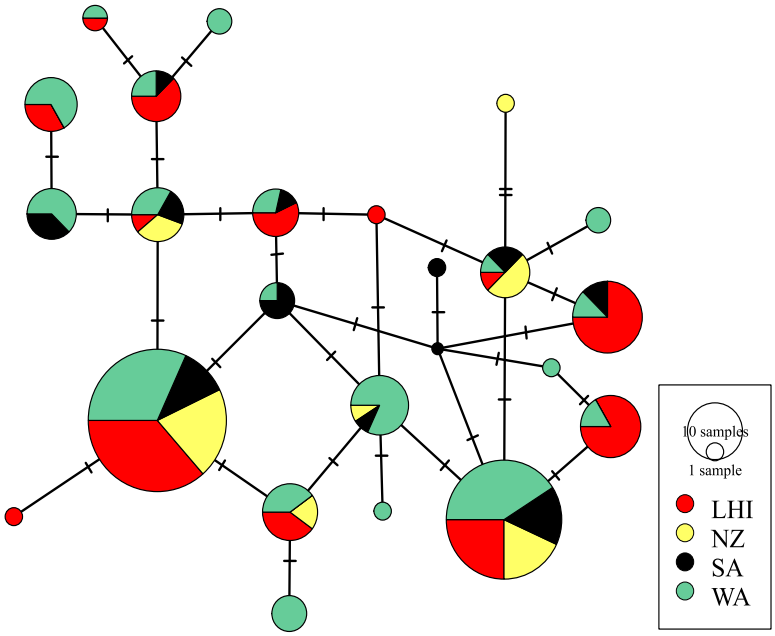
	Lord Howe Island		New Zealand		South Australia		Western Australia		Saint-Paul Island		Sea of Japan		All individuals	
Locus	*D**	*D*	*D**	*D*	*D**	*D*	*D**	*D*	*D**	*D*	*D**	*D*	*D**	*D*
Cyt b	-0.849	-1.302	-0.535	0.559	-1.159	-1.377	-0.239	-0.303	–	–	-0.535	-0.901	-2.269	-0.119
4080	0.617	1.261	-0.363	1.026	1.214	1.681	0.302	0.302	–	–	–	–	-0.421	0.250
18503	0.199	0.909	0.706	0.665	1.138	1.696	1.139	2.098*	–	–	–	–	0.183	1.596
20454	1.471	3.977***	1.512*	2.474*	1.505*	3.131**	1.003	3.696***	–	–	–	–	0.848	4.365***
22519	0.844	0.877	-0.008	-0.273	-0.688	-0.688	-0.376	-0.376	–	–	–	–	0.153	-0.492
Pema01	-0.508	0.464	1.439	-1.008	-1.041	-0.768	0.864	0.046	–	–	–	–	-1.314	-0.398
Pema07	-1.019	-1.055	-1.599	-0.177	-1.102	0.132	-1.019	-0.278	–	–	–	–	-1.507	-0.769
Pema14	-0.539	-1.276	0.394	-0.196	-1.358	-0.981	-0.519	-1.469	–	–	–	–	-0.089	-1.637

*p <0.05, **p < 0.01, ***p <0.001

4080

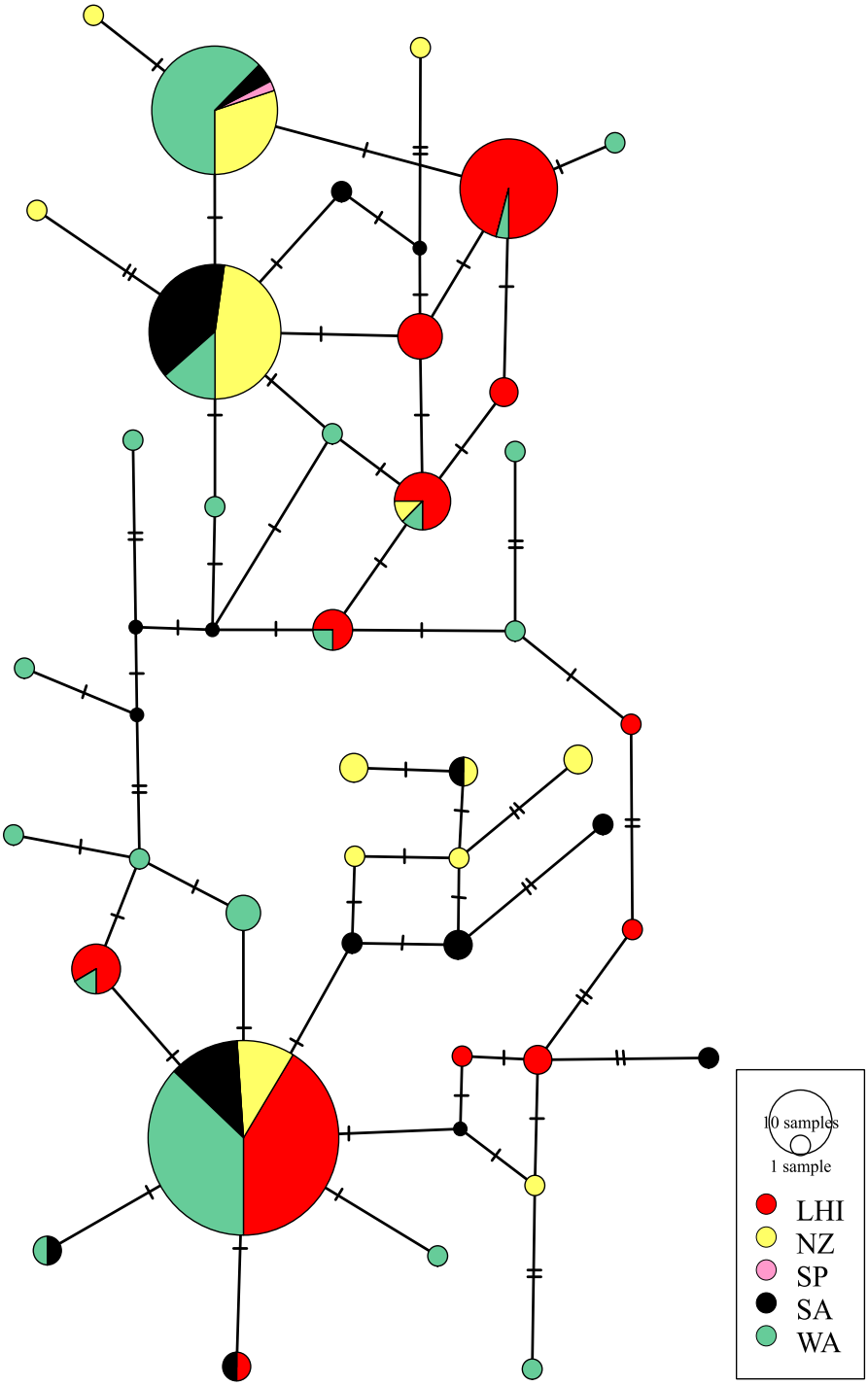


18503

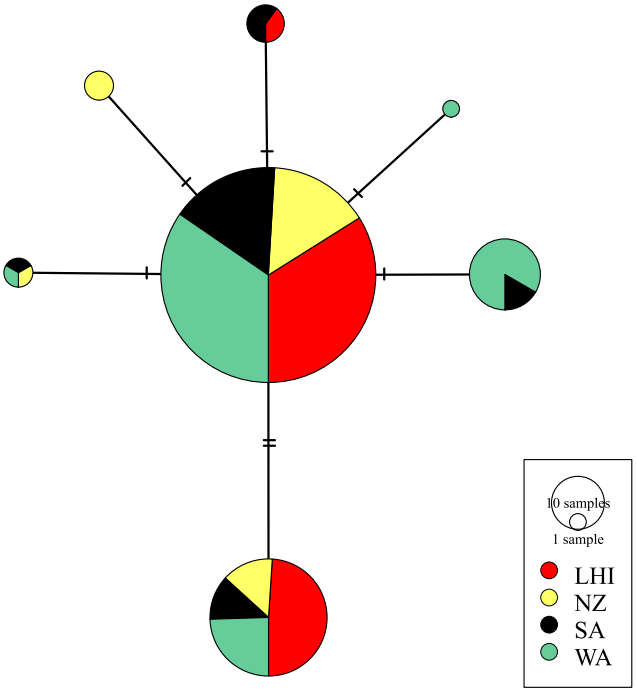


SI. 3 – 7 Haplotype networks of seven *A. carneipes* nuclear DNA fragments based on the TCS algorithm. Haplotypes are represented by circles, where the size of each circle is proportional to the frequency of the corresponding haplotype. Lines on connecting branches represent one single mutation. Figure continues over successive pages.

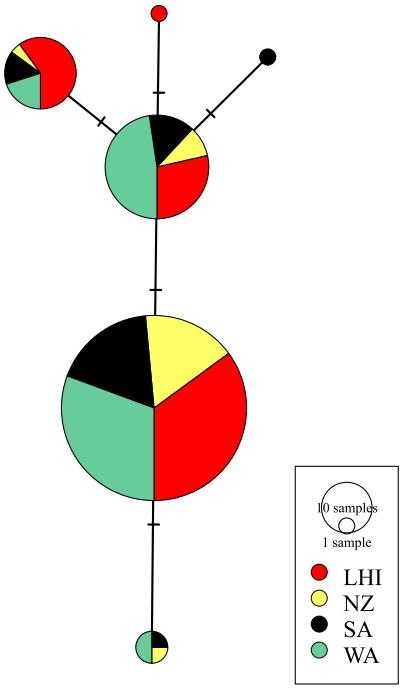
20454



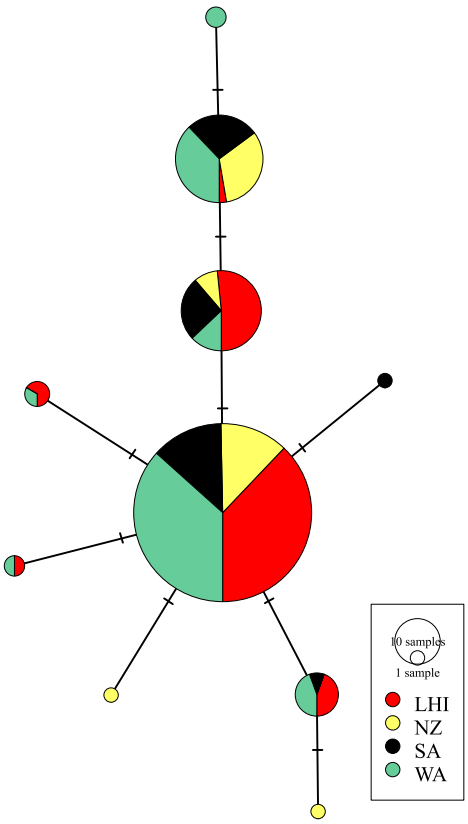
22519



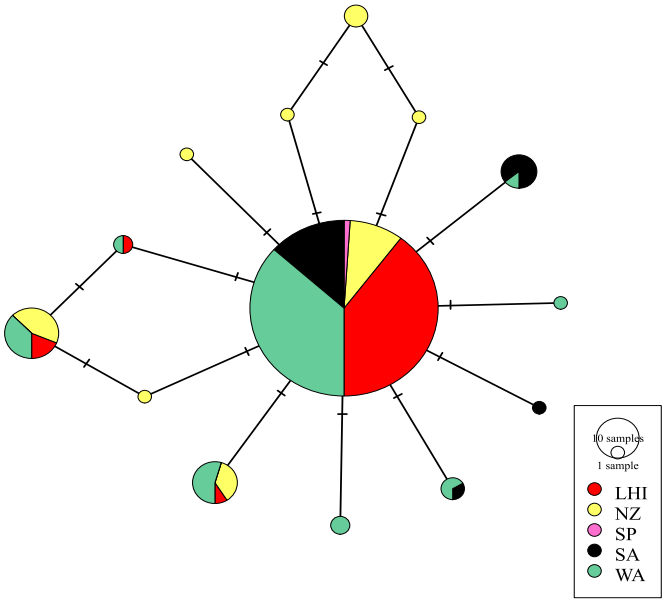
Pema01



Pema07



Pema14



SI. 3 – 8 AMOVA Φ-statistics (Φ_{SC} Φ_{ST} Φ_{CT}) (Excoffier *et al*., 1992) for *A. carneipes* breeding regions based on Cytochrome *b*. A total of seven (*K*=2), six (*K*=3) and one (*K*=4) groupings of breeding regions were tested. *p*-values for Φ_{SC} are based on permutations of sampled sequences across regions within the same group, *p*-values for Φ_{ST} are calculated based on permutations of sampled sequences among regions without regard to their original group, *p*-values for Φ_{CT} are based on permutations of whole regions among groups.

Groups	Φ_{SC}	Φ_{ST}	Φ_{CT}
***K*=2**			
[LHI, NZ][SA, WA]	0.239*	0.782*	**0.714***
[LHI, SA][NZ, WA]	0.785*	0.674*	-0.519
[LHI, WA][SA, NZ]	0.787*	0.656*	-0.062
[LHI] [NZ, SA, WA]	0.681*	0.741*	0.188*
[NZ] [LHI, SA, WA]	0.761*	0.655*	-0.435
[SA] [LHI, NZ, WA]	0.705*	0.749*	0.151
[WA] [LHI, NZ, SA]	0.717*	0.722*	0.016
***K*=3**			
[LHI][NZ][SA, WA]	0.139*	0.743*	**0.702***
[LHI][SA][NZ, WA]	0.720*	0.720*	0.003
[LHI][WA][NZ, SA]	0.798*	0.712*	-0.433
[NZ][SA][LHI, WA]	0.782*	0.683*	-0.045
[NZ][WA][LHI, SA]	0.841*	0.691*	-0.942
[SA][WA][LHI, NZ]	0.296*	0.756*	0.653*
***K*=4**			
[LHI][NZ][SA][WA]	-	**0.519***	-

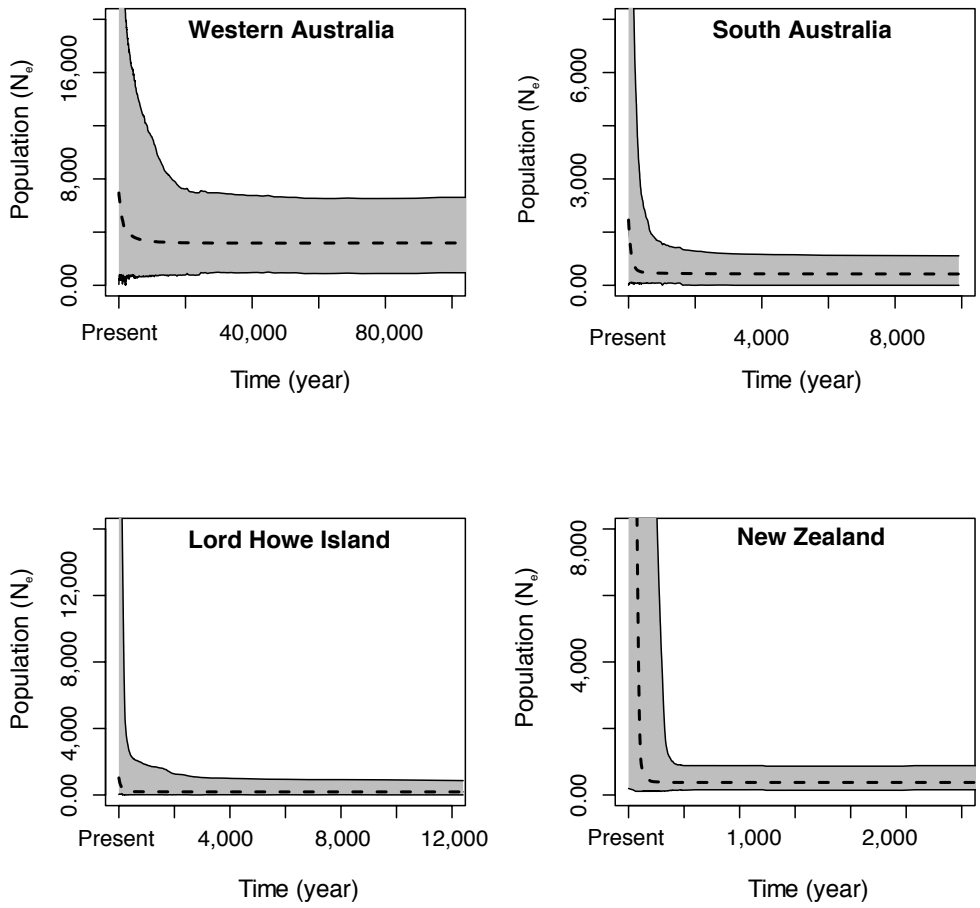
*$p<0.05$

SI. 3 – 9 Marginal posterior distribution of the parameters for the Isolation with Migration model estimated for eastern and western *A. carneipes* breeding colonies (*K* = 2) as implemented in IMa.

	θ (4Nμ)		*m*		*T* (years)
Eastern colonies	3,800 (2,000 – 5,000 90% HPD)	East > West	~0 (9 E^{-4} – 2 E^{-3} 90% HPD)	East – West	28,000 (11,700 – 100,000 90% HPD)
Western colonies	4,500 (2,800 – 7,500 90% HPD)	West > East	~0 (7 E^{-4} – 4 E^{-3} 90% HPD)		

SI. 3 –10 Topology-1/Topology-2. Marginal posterior distribution of the genetic parameters for the Isolation with Migration model among Lord Howe Island, New Zealand, South Australia and Western Australia *A. carneipes* breeding regions (*K*=4) as implemented in IMa2 including Topology-1 and Topology-2. Shaded = best genetic clustering (AMOVA).

	θ (4Nμ)		*m*		*T* (years)
Topology-1					
Lord Howe Island (LHI)	4,000 (1,300 – 13,000 90% HPD)	LHI > NZ	~0 (2.E^{-5} – 7.E^{-4} 90% HPD)	[LHI-NZ] – [SA-WA]	28,000 (9,800 – 76,000 90% HPD)
New Zealand (NZ)	5,600 (3,500 – 12,000 90% HPD)	NZ > LHI	~0 (4 E^{-5} – 7 E^{-4} 90% HPD)	[LHI] – [NZ]	3,000 (2,000 – 7,000 90% HPD)
South Australia (SA)	2,500 (1,000 – 13,000 90% HPD)	SA > WA	~0 (2 E^{-4} – 7 E^{-4} 90% HPD)	[SA] – [WA]	2,100 (800 – 6,000 90% HPD)
Western Australia (WA)	3,000 (1,400 – 6,000 90% HPD)	WA > SA	~0 (1 E^{-4} – 7 E^{-4} 90% HPD)		
Topology-2					
South Australia (SA)	5,000 (2,600 – 13,000 90% HPD)	SA > WA	~0 (1.E^{-6} – 2.E^{-4} 90% HPD)	[SA-WA] – [LHI-NZ]	28,500 (13,000 – 95,500 90% HPD)
Western Australia (WA)	6,000 (2,500 – 13,000 90% HPD)	WA > SA	~0 (4 E^{-6} – 2 E^{-4} 90% HPD)	[SA] – [WA]	3,000 (1,400 – 7,000 90% HPD)
Lord Howe Island (LHI)	3,000 (1,300 – 11,000 90% HPD)	LHI > NZ	~0 (5 E^{-6} – 2 E^{-4} 90% HPD)	[LHI] – [NZ]	2,000 (800 – 6,000 90% HPD)
New Zealand (NZ)	3,000 (1,500 – 5,000 90% HPD)	NZ > LHI	~0 (5 E^{-6} – 2 E^{-4} 90% HPD)		



SI. 3 – 11 Extended Bayesian Skyline Plot (EBSP) showing demographic reconstruction of *A. carneipes* individuals. Four regions (Lord Howe Island, New Zealand, West Australia and South Australia) through time for Cytochrome *b* and four nuclear DNA fragments after having discarded the three nuclear loci suspected to have experienced recombination (*4080, 18503, 20454*).

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Chapter 4:

Population genetic and behavioural variation of the two remaining colonies of providence petrel (*Pterodroma solandri*)

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Population genetic and behavioural variation of the two remaining colonies of providence petrel (*Pterodroma solandri*)

Anicee J. Lombal[1,5], Theodore J. Wenner[1,6], Nicholas Carlile[2,7], Jeremy J. Austin[3,8], Eric Woehler[4,9], David Priddel[2,10] and Christopher P. Burridge[1,11].

[1]School of Biological Sciences, University of Tasmania, Hobart, TAS 7001, Australia. Phone: +61 3 6226 7653.

[2]Department of Environment and Conservation, P.O. Box 1967, Hurtsville, NSW 2220, Australia. Phone: +61 2 9585 6554

[3]Australian Centre for Ancient DNA, School of Biological Sciences, University of Adelaide, Adelaide, SA 5005, Australia. Phone: +61 8 8313 4557

[4]Institute for Marine and Antarctic Studies, University of Tasmania, Hobart, TAS 7053, Australia. Phone: +61 3 6226 6379

[5]Anicee.Lombal@utas.edu.au

[6]Theodore.Wenner@utas.edu.au

[7]Nicholas.Carlile@environment.nsw.gov.au

[8]Jeremy.austin@adelaide.edu.au

[9]Eric.woehler@utas.edu.au

[10]David.priddel@environment.nsw.gov.au

[11]Chris.Burridge@utas.edu.au

Keywords: oceanic seabird, *Pterodroma solandri*, gene flow, behavioural variation, conservation management

Abstract

Knowledge of the dispersal capacity of species is crucial to assess their extinction risk, and to establish appropriate monitoring and management strategies. The providence petrel (*Pterodroma solandri*) presently breeds only at Lord Howe Island (~32,000 breeding pairs) and Phillip Island – 7km south of Norfolk Island (~20 breeding pairs). A much larger colony previously existed on Norfolk Island (~1,000,000 breeding pairs) but was hunted to extinction in the 18th Century. Differences in time of return to nesting sites are presently observed between the two extant colonies. Information on whether the Phillip Island colony is a relict population from Norfolk Island, or a recent colonization from Lord Howe Island, is essential to assess long-term sustainability and conservation significance of this small colony. Here, I sequenced the mitochondrial cytochrome *b* gene and 14 nuclear introns, in addition to genotyping 10 microsatellite loci, to investigate connectivity of the two extant *P. solandri* populations. High gene flow between populations and recent colonization of Phillip Island (95% HPD 56–200 ya) are inferred, which may delay or prevent the genetic differentiation of these insular populations. These results suggest high plasticity in behaviour in this species and imply limited genetic risks surrounding both the sustainability of the small Phillip Island colony, and a proposal for translocation of Lord Howe Island individuals to re-establish a colony on Norfolk Island.

Introduction

Understanding mechanisms of population divergence has important implications for successful conservation of species (Avise, 2000). While adaptation to different environments may be important for population persistence, it may also inhibit movements amongst populations, potentially reducing genetic variability through random genetic drift and inbreeding (Frankham, 1996; Hedrick and Kalinowski, 2000), which may decrease adaptability to future environmental variations (Frankham *et al*., 2002). Therefore, quantifying the dispersal of individuals, which is driven by the variability in intrinsic patch quality between different areas such as resource availability or population density (Bowler and Benton, 2005), is essential to predict the long-term resilience and persistence of populations, and to inform management decisions such as supplementation and translocation (Frankham, 1996).

Seabirds provide useful model systems for studying mechanisms of population divergence given their often philopatric behaviour and discrete breeding distributions (Friesen *et al*., 2007; Friesen, 2015). Most oceanic seabirds breed in discrete colonies, and may constitute a population structure known as metapopulations, where occasional dispersal facilitates re-establishment or supplementation of populations following declines (Oro, 2003). Nevertheless, the relative importance of the factors influencing dispersal between seabird colonies remains unclear (Friesen, 2015). While physical barriers to dispersal and philopatry appear to be the main inhibitors of gene flow among seabird colonies (Friesen 2015; Warham 1990), other mechanisms have also been detected, such as differences in foraging distribution during the breeding and post-breeding seasons, differences in ocean regimes, and differences in breeding phenology (Burg and Croxall, 2001; Wiley *et al*., 2012). For example, allochronic populations of band-rumped storm-petrel (*Oceanodroma castro*) appear

genetically isolated in five archipelagos throughout the Atlantic and Pacific Oceans in the absence of physical barriers to gene flow (Smith and Friesen, 2007). Conversely, whether genetic isolation exists among colonies that exhibit other phenological or circadian differences, e.g. diurnal vs. nocturnal colony attendance has yet to be investigated.

The providence petrel (*Pterodroma solandri*) is classified as vulnerable under both the *IUCN Red List of Threatened Animals* (Criteria D2) and the *New South Wales Threatened Species Conservation Act 1995* due to its restricted breeding range. The only significant breeding locality is Lord Howe Island (~32,000 breeding pairs) (Bester, 2003), a small island located 600 km off the eastern coast of Australia (Figure 4 – 1). providence petrels previously bred on Norfolk Island (~1,000,000 breeding pairs), located approximately 1100 km northeast of Lord Howe Island (Figure 4 – 1), before becoming extirpated following European settlement by the late 18th century (Medway, 2002a). This species was considered extinct within the Norfolk Island group until 1986 when a small population (~20 breeding pairs) was discovered on Phillip Island, 7 km south of Norfolk Island (Hermes *et al*., 1986) (Figure 4 – 1).

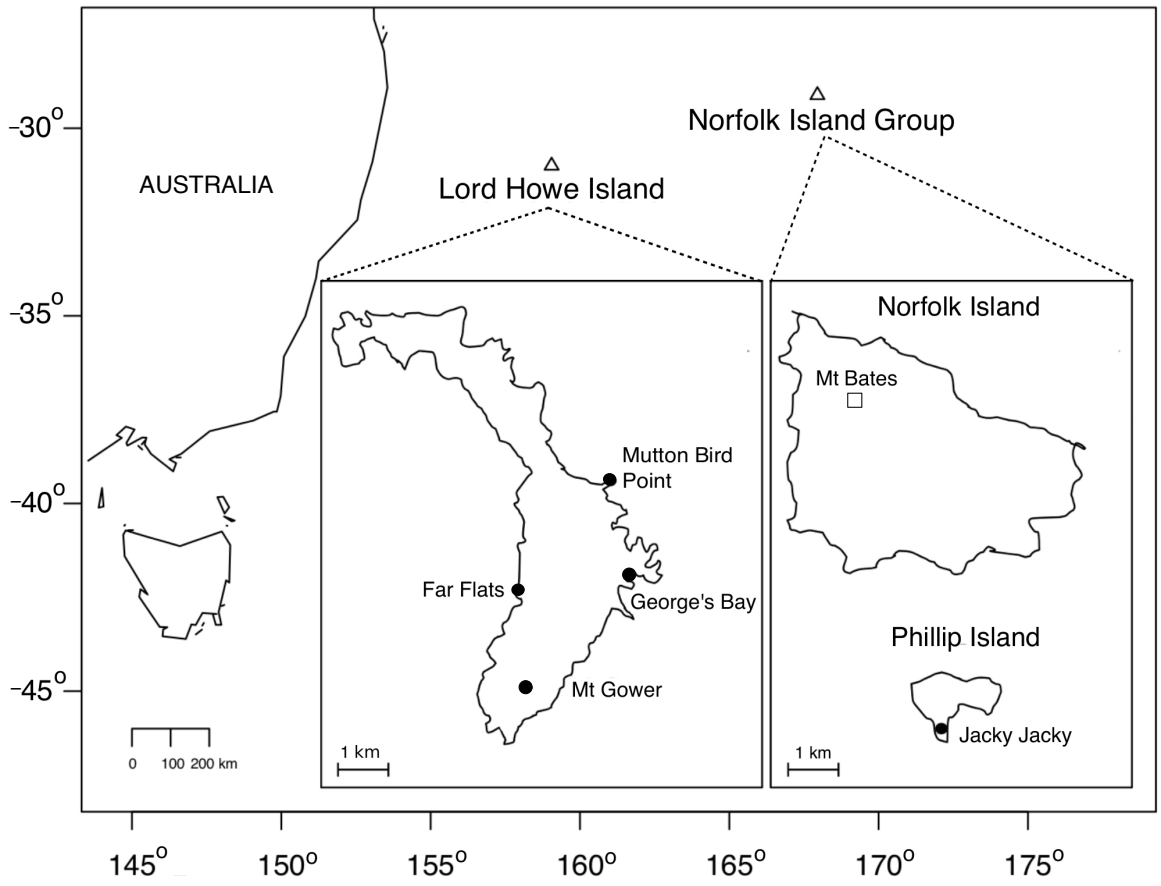

Figure 4 – 1 Sampling locations for *Pterodroma solandri*. Lord Howe Island: Far Flats (FF, n=79), George's Bay (GB, n=22), Muttonbird Point (MBP, n=20), Mount Gower (MG, n=30). Phillip Island: Jacky Jacky (JJ, n=32). Mount Bates was the location of the extinct Norfolk Island colony.

There is no evidence justifying taxonomic separation between Phillip Island and Lord Howe Island providence petrels. However, it has been reported that Lord Howe Island individuals predominantly arrive at the colony during daylight (Bester *et al*., 2002; Medway, 2002b), while Phillip Island individuals return to their breeding sites only after dusk (pers. obs.). This may relate to the presence of diurnal aerial predators - brown goshawks *Accipiter fasciatus* - at the time of European settlement on Norfolk Island (Medway, 2002b), although no such predation risk presently exists. Alternatively, differences in foraging areas may explain time of return to colony (Dias *et al*., 2012). Given the possibility of selective significance, the observed difference in behaviour between colonies may inhibit gene flow between them.

Here I report a comprehensive study of the genetic distinctiveness between the two remaining breeding colonies of providence petrel, to infer the dispersal patterns of this species and the conservation status of the small Phillip Island colony. I developed three genetic data sets, consisting of DNA sequences from mitochondrial and 14 nuclear regions and genotypes from 10 microsatellite loci, to investigate genetic connectivity and evolutionary history of providence petrel colonies. This study is also relevant to the proposed re-establishment of a colony on Norfolk Island using individuals from Lord Howe Island, with the aim of reducing the extinction risk of this species, and restoring the input of marine-derived nutrient into the ecosystem.

Materials and Methods

Sample collection and DNA extraction

I collected blood samples from *P. solandri* individuals (n = 151) from four localities on Lord Howe Island (31°30'S, 159°05'E): Mount Gower (MG n = 30), Far Flats (FF n = 79), George's Bay (GB n = 22) and Muttonbird Point (MBP n = 20) (Fig.1). I sampled the one

locality on Phillip Island (29°12'S, 167°95'E) where the providence petrel has been observed to nest: Jacky Jacky (JJ $n = 32$) (Figure 4 – 1). All blood samples were collected from providence petrels under Animal Ethics Permit number AEC 021028/02 issued by the Department of Environment, Climate Change and Water (NSW). Genomic DNA was extracted from 183 individuals using a Qiagen DNeasy® Blood and Tissue kit following the manufacturer's protocol.

Mitochondrial and nuclear DNA sequencing

I sequenced 183 individuals (151 from Lord Howe Island, 32 from Phillip Island) for a 872 bp fragment of the mitochondrial cytochrome *b* gene using primers L14841 (Kocher *et al*., 1989) and H15547 (Edwards *et al*., 1991). I also sequenced 40 individuals (20 from FF, Lord Howe Island, 20 from JJ, Phillip Island) for ~500 bp long fragments of 14 avian nuclear introns (Backström *et al*., 2008; Patterson *et al*., 2011; Silva *et al*., 2011). Primer sequences, optimal annealing temperatures and approximate locus length in *P. solandri* are shown in the Supplementary Information, SI. 4 – 1.

All fragments were PCR amplified with the MangoTaq™ DNA polymerase following the manufacturer's protocol (Bioline Inc.). PCR reactions were performed in 35 μL volumes using 50–100 ng DNA, and final concentrations of 0.5 U DNA polymerase, 0.2 mM of each dNTP, 1.5 mM $MgCl_2$ and 0.3 μM of each primer. The thermal cycling profiles included an initial denaturation at 95°C for 1 min followed by 29 cycles of 95°C for 30 s, 60–46°C (decreasing the annealing temperature by 0.5°C per cycle) for 40 s, and an extension of 72°C for 90 s, with a final extension of 72°C for 10 min followed by four similar cycles but with a

constant annealing temperature at 45°C. Negative controls were included with each set of PCRs.

Nucleotide sequences were determined on both strands of PCR products using a 3730xl DNA Analyzer (Applied Biosystem®) at Macrogen Inc., Korea. Sequences were aligned using the MUSCLE algorithm (Edgar, 2004) in CODONCODE ALIGNER v3.7.1.1 (CodonCode Corporation). For sequences containing multiple heterozygous positions, I used the maximum likelihood method implemented in PHASE v2.2.1 (Stephens *et al*., 2001) to reconstruct the haplotype phase of the sequences. I conducted three independent runs of 10,000 iterations per locus with a different seed number to verify convergence, and discarded the first 1,000 samples as burn-in. Phased haplotypes showing a probability >0.8 were used for further analyses.

Microsatellite genotyping

Genotypes of 183 individuals (151 from Lord Howe Island, 32 from Phillip Island) were determined at 10 polymorphic microsatellite loci by capillary electrophoresis (*Ptero9*, *Ptero7*, *Ptero6*, *Ptero4*, *Parm02*, *Parm03*, *Paequ03*, *Paequ13*, *Calex01*, *RBG29*) following Lombal et al, (2015) (see Chapter 2).

Data analyses

Tests of assumptions and genetic variation

To assess levels of DNA sequence variation within colonies (Lord Howe Island, Phillip Island), haplotypic diversity *h* (Nei, 1987), haplotype ratios X_H, nucleotide diversity π (Tajima, 1983), and nucleotide diversity ratios π_R (Mardulyn *et al*., 2009) were calculated for mitochondrial and nuclear intron DNA sequences with SPADS v 1.0 (Dellicour and Mardulyn, 2014). To test whether patterns of genetic variation deviated from neutral expectations, Tajima's *D* test (Tajima, 1989) and Fu and Li's *D** test (Fu and Li, 1993) were performed using DNASP v 5.10 (Librado and Rozas, 2009).

Microsatellite loci were tested for departure from Hardy-Weinberg equilibrium for each colony (Lord Howe Island, Phillip Island) using exact tests in ARLEQUIN v 3.5.1.2 (Excoffier and Lischer, 2010), where Markov chain parameters were set at 10,000 dememorizations, and 10,000 iterations. The inbreeding coefficient F_{is} ($1 - H_o/H_E$) was calculated per colony in FSTAT 2.9.2. (Goudet, 1995), then tested for significant departure from zero using 10,000 permutations of alleles among individuals. Allelic diversity N_a, and allelic richness R_s, which uses a rarefaction method to standardize uneven sample size (Petit *et al*., 1998), were computed with the software HP-RARE v 1.0. (Kalinowski, 2005).

Population connectivity and identification of dispersers

Estimates of pairwise population differentiation between Lord Howe Island and Phillip Island (F_{st}, G_{st}, N_{st} and Φ_{st}) were determined using SPADS v 1.0 (Dellicour and Mardulyn, 2014). The statistical significance of F_{st}, G_{st}, N_{st} and Φ_{st} values was assessed by recalculating them based on 10,000 random permutations of individuals among islands. TCS networks (Clement *et al*., 2000) were inferred for mitochondrial and nuclear DNA sequences using PopART (http://popart.otago.ac.nz). AMOVA Φ-statistics (Φ_{SC} Φ_{ST} Φ_{CT}) (Excoffier *et al*., 1992) were calculated for the mitochondrial locus (cyt *b*) (Group 1= JJ, Phillip Island; Group 2 = FF, MBP, MG, GB, Lord Howe Island) with 10,000 permutations of individuals and sampling sites. In addition, to evaluate the extent to which sequence variation was partitioned, a matrix of pairwise population differentiation was constructed between all sampling sites (n = 5).

F_{st} and R_{st} (Slatkin, 1995) were calculated for microsatellites, the latter assuming a generalized stepwise mutation model (SMM), using FSTAT 2.9.2 (Goudet, 1995), with significance assessed based on 10,000 permutations of alleles among samples. Contingency tables of alleles were generated, and classified (Kimura and Ohta, 1978) using the log-likelihood statistic G (Goudet *et al*., 1996). G_{st} were not calculated for these high mutation rate markers as recommended by Whitlock (2011). AMOVA (Excoffier *et al*., 1992) was performed with 10,000 permutations of individuals among sampling sites (Group 1= JJ, Phillip Island; Group 2 = FF, MBP, MG, GB, Lord Howe Island), and a pairwise population differentiation matrix was constructed among all sampling sites (n = 5) using GENODIVE v 2.0b28 (Meirmans and Van Tienderen, 2004).

As low genetic divergence among populations could reflect high historical dispersal among populations that are now isolated, I used kinship-based methods to estimate current gene flow

between *P. solandri* colonies (Lord Howe Island, Phillip Island), as recommended when there are low frequency alleles present (Hardy and Vekemans, 2002). The statistical rigor and power of this approach using kinship coefficients (θ_{ij}) depends upon the overall level of genetic variation, and not the degree of divergence between populations (Palsboll *et al*., 2010). I calculated θ_{ij} (Loiselle *et al*., 1995) for each pair of individuals in GENODIVE (Meirmans and Van Tienderen, 2004). To test whether individuals collected in the same colony were more closely related to each other than individuals collected in different colonies, I performed a non-parametric Permutational Multivariate Analysis of Variance (PERMANOVA) on θ_{ij}. This approach partitions the distance matrix according to the source of variation (e.g. among vs. within), and compares the sum of square distances among and within these groups as implemented in PERMANOVA+ 1.0.6 software add-on running on PRIMER6 (Clarke and Warwick, 2005). To assign θ_{ij} to independent genetic clusters, I used a *K*-Means method to calculate the Calinski-Harabasz pseudo *F*-statistics (Caliński and Harabasz, 1974), which focuses on reducing the within-group sum of squares, for $K = 2–183$, with 10,000 iterations per cluster, as implemented in the package *clusterSim* in R v 3.2.1.

Bayesian clustering analysis and individual assignment

Bayesian clustering analysis, which uses MCMC simulation to assign coancestry of individuals to independent genetic clusters (*K*) based on individual microsatellite genotypes without *a priori* assumptions of populations, was implemented in STRUCTURE v 2.3.3 (Pritchard *et al*., 2000; Falush *et al*., 2003). Exploratory runs showed that a burn-in of 200,000 followed by 1,000,000 iterations achieved stable estimates. 20 replicate runs were then performed for all values of $K=1–8$, reflecting the highest expected number of genetic cluster ($n = 5$, Far Flats (FF), Mount Gower (MG), George's Bay (GB), Muttonbird Point (MP), and Jacky Jacky (JJ)) plus three (Evanno *et al*., 2005). I used the admixture model, and

assumed correlated allele frequencies, which is expected to perform better when genetic structure is weak or when the number of loci is < 20 (Hubisz *et al*., 2009), with Prior Mean = 0.01, and Prior SD = 0.05. I implemented priors for alpha ($\alpha = 1$) and lambda ($\lambda = 1$), specifying the degree of admixture between populations and the distribution of allele frequencies respectively, for all populations. The optimal number of clusters (*K*) was estimated by calculating the second order-rate of change (ΔK) of the likelihood function (ln $P(X/K)$) with respect to each *K* (Evanno *et al*., 2005), as implemented in the program STRUCTURE HARVESTER (Earl, 2012). The results of all runs were summarized in CLUMPP v 1.1.1 (Jakobsson and Rosenberg, 2007) using the *FullSearch* algorithm, and then visualized using DISTRUCT v 1.1 (Rosenberg, 2004).

Migrant individuals between colonies (Lord Howe Island, Phillip Island) were identified using exclusion methods as implemented in GENECLASS 2.0 (Piry *et al*., 2004). I used the exclusion criterion L_h/L_{max} (Paetkau *et al*., 2004) to compute the probability that an individual belongs to a colony. I compared the Bayesian (Rannala and Mountain, 1997) and frequency based criteria (Paetkau *et al*., 2004) to calculate the likelihood of individual origin. I used the Paetkau (2004) resampling methods based on allele frequency (Paetkau *et al*., 2004), which demonstrated low type I error rates (1% of the number of individuals per population that appear to be immigrants by chance). This method generates population samples of the same size as the reference population sample, as recommended for detection of first generation migrants (Piry *et al*., 2004). The marginal probability of given individual multilocus genotype was compared to the distribution of marginal probabilities of randomly generated multilocus genotypes (100,000 replicates) with a type I error threshold setting at $\alpha_{0.01}$ and $\alpha_{0.05}$.

Estimation of divergence time

I used IMa and its model of isolation with migration (Hey and Nielsen, 2007) to simultaneously estimate migration (m_1, m_2) and lineage divergence time (*t*) between *P. solandri* colonies (Lord Howe Island, Phillip Island). This coalescent-based model is based on several assumptions including neutrality, random mating in ancestral and descendent populations, and free recombination between loci, but none within loci (Nielsen and Wakeley, 2001; Hey and Nielsen, 2004). Lack of recombination within nuclear introns was tested using the four-gamete test as described by Hudson and Kaplan (1985), and loci suspected to be under selection were excluded from analyses (Supplementary Information, SI. 4 – 3). An IMa exploratory run was performed to assess a range of prior distributions that include most of the range over which the posterior density is not trivial. Analyses were then run three times with different seed numbers to test for convergence, with 10,000,000 sampled steps following a discarded burn-in of 200,000 steps, with a two-step linear heating scheme with five chains. I implemented the Hasegawa-Kishino-Yano (HKY) (Hasegawa *et al*., 1985) model for the mitochondrial data, the infinite sites mutation model (IS) (Kimura, 1969) for the nuclear introns, and the Stepwise Mutation Model (SMM) (Kimura and Ohta, 1978) for microsatellites. Mutation rates were given as priors to the analysis with $\mu = 1.89 \times 10^{-8}$ and 3.6×10^{-9} substitution/site/year for cyt *b* and nuclear introns respectively, as recommended for other seabirds (Axelsson *et al*., 2004; Weir and Schluter, 2008), and $\mu = 5 \times 10^{-4}$ substitution/site/year for microsatellites (Brown *et al*., 2010). To assess the estimates of demographic parameters, I used a generation time T = 10 years, as calculated based on the following equation T = A+ p/(1-p) (Sæther *et al*., 2004), with p the adult survival rate (p = 0.82) (Brooke, 2004), and A the age of sexual maturity (A = 6 years) (Warham, 1990). Parameter trend line plots and values of effective sample sizes (ESS) were inspected after each run.

Demographic history

Historical demographic changes in the only significant colony of providence petrels (Lord Howe Island) were inferred from two complimentary coalescent modeling approaches of microsatellite data using MSVAR v0.4 (Beaumont, 1999) and MSVAR v1.3 (Storz and Beaumont, 2002). This approach is more robust than classic methods based on summary statistics to detect changes in population size (Girod *et al.*, 2011).

MsVar v0.4 inferred the magnitude of change in population size ($r = N_0/N_1$, where N_0 and N_1 are current and ancestral population sizes, respectively) assuming a SMM model for the microsatellite loci. I initially conducted three independent simulations varying the prior distributions to examine their effect on the posterior distribution. I then ran the simulation three times under the exponential and the linear model, with different seed numbers for each dataset, for 4×10^9 iterations with parameter values recorded every 1×10^5 iterations, resulting in 40,000 records. I discarded 10% of recorded values for each chain (i.e. burn-in), and I performed the Brooks, Gelman and Rubin Convergence diagnostic tests (Gelman and Rubin, 1992) as implemented in the package BOA (Smith, 2007) for R version 3.2.1. (Venables *et al.*, 1998). I considered that chains converged well when values lower than 1.1 were obtained. The chains were then combined to estimate the 90% high probability density (HPD) of demographic parameters using the package CODA as implemented in R (Plummer *et al.*, 2006). The strength of evidence for population increase versus decrease was evaluated by calculating the Bayes factor of each of the simulations (Storz *et al.*, 2002; Girod *et al.*, 2011). This ratio can be estimated by counting the number of states in the chains in which the population has decreased (i.e. $N_0/N_1<1$), and then dividing this by the number of states in which the population has increased (i.e. $N_0/N_1>1$) with BF 0 – 3 no support of contraction, 3 – 10 substantial support, >10 strong support (Storz *et al.*, 2002).

Msvar 1.3 was used to quantify population sizes and time of change. MsVar 1.3 uses probable genealogies of allele frequency data to generate posterior probability distributions of four natural demographic parameters, $\Phi = N_0, N_1, ta,$ and θ, where N_0 and N_1 are the current and the ancestral effective population size respectively, *ta* is the time since the demographic changes began, and $\theta = 4N_0\mu$, the rate of mutation scaled by population size. This model differs from the previous model in that all loci are used in the same MCMC simulation, reducing density estimation error, and that all parameters are free to vary among loci. I inferred broad normal distribution priors and hyperpriors (Supplementary Information, SI. 4 – 2), and I ran the simulation three times under the exponential model to evaluate recent changes in population size ($\log_{10}(T)<10$). MCMC chain convergence, 90% HPD of posterior distributions and Bayes factors were inferred as described for Msvar v0.4.

Results

I sequenced 872 bp of the mtDNA cytochrome *b* gene in 151 and 32 individuals from Lord Howe Island and Phillip Island, respectively, and a total of 7837 bp comprising 14 nuclear introns in 20 individuals from both colonies, defining 2–9 (Phillip Island) and 1–17 (Lord Howe Island) alleles (Supplementary Information SI. 4 – 3). No significant difference in nucleotide diversities (π) between colonies was detected (One-way ANOVA; H_0= means of π_R are equal in Lord Howe Island and Phillip Island, where π_R represents the nucleotide ratio; F = 0.91; *p*-value = 0.349; see π_R values in Supplementary Information SI. 4 – 3). Tajima's *D* statistics showed significant negative values in the mitochondrial locus (*cyt b*, *D* = -1.987, p <0.05), and in one nuclear intron (*δ-cryst*, *D* = -2.030, p<0.05) for Lord Howe Island and Phillip Island populations respectively, while Fu and Li's *D** tests showed negative values for two loci (*cyt b*, *D** = -2.920, p<0.05, and *16214*, *D** = -3.110, p<0.05) for Lord Howe

Island, and in one locus (*Pema05*, D^* = -2.167, $p<0.05$) for Phillip Island (Supplementary Information SI. 4 – 3).

Ten microsatellite loci were genotyped in 151 and 32 individuals from Lord Howe Island and Phillip Island, defining 2–51 and 4–30 alleles per locus, respectively. No significant difference in genetic diversity between populations was detected (Kruskal-Wallis test; H_0= means of R_s are equal in Lord Howe Island and Phillip Island, where R_s represents allelic richness; F = 1.12; p-value = 0.289; see R_s values in Table 4 – 1), and no significantly positive values of F_{is} were found for either Lord Howe Island or Phillip Island (Table 4 – 1).

Population connectivity

Visual inspection of haplotype networks (Figure 4 – 2; Supporting Information SI. 4 – 4), observation of low *F*-statistics (global F_{st} = 0.004, $p>0.05$; global G_{st}= 0.004, $p>0.05$; Table 4 – 2) and lack of significant phylogeographic signals (global Φ_{st} = 0.019, $p>0.05$, global N_{st}= 0.033, $p>0.05$; Table 4 – 2) indicate no genetic differentiation between Lord Howe Island and Phillip Island. AMOVA Φ-statistics showed no differentiation between sampling locations or group of sampling locations for cyt *b* (Group 1= JJ, Phillip Island; Group 2 = FF, MBP, MG, GB, Lord Howe Island; Φ_{sc} = 0.0004, $p>0.05$; Φ_{st} = 0.016, $p>0.05$; Φ_{ct}= 0.016, $p>0.05$), and the F_{st} pairwise matrix showed no significant genetic structure between pairs of *P. solandri* sampling sites (Table 4 – 3). F_{st}, R_{st} and AMOVA *F*-statistics obtained with microsatellites were not significantly different from zero between Phillip Island and Lord Howe Island sampling locations (global F_{st}= 0.006, $p>0.05$; global R_{st}= 0.004, $p>0.05$; Table 4 – 1). Pairwise F_{st} indicated no genetic differentiation between *P. solandri* sampling sites (Table 4 – 3). These results refute structuring of genetic variation between Lord Howe Island and Phillip Island.

Table 4 – 1 Characterization of genetic diversity and summary statistics in *P. solandri* for 10 microsatellites loci. Allelic diversity (A), allelic richness (R_s) and tests for departure from Hardy-Weinberg equilibrium. Inbreeding coefficient F_{is} ($1 - H_o/H_E$). Population structuring (F_{st} and R_{st}).

		Lord Howe Island (n = 151)					Phillip Island (n = 32)					All populations		
Locus name	Length (bp)	A	R_s	H_o	H_e	F_{is}	A	R_s	H_o	H_e	F_{is}	F_{st}	R_{st}	F_{is}
Ptero09	187-235	17	14.47	0.671	0.879	0.234	13	10.93	0.688	0.886	0.227	-0.004	0.024	0.232
Ptero07	264-344	51	41.26	0.968	0.954	-0.008	30	21.25	1.000	0.972	-0.029	-0.007	0.002	-0.010
Parm03	177-181	6	5.00	0.654	0.663	0.011	4	3.75	0.469	0.637	0.268	0.004	0.024	0.055
Ptero06	141-149	2	2.00	0.033	0.185	0.821	3	2.94	0.156	0.347	0.553	0.038	-0.016	0.746
Paequ13	146-148	5	4.20	0.266	0.352	0.239	4	3.023	0.355	0.421	0.159	0.006	0.004	0.222
Calex01	237-255	14	13.75	0.859	0.859	-0.002	13	11.33	0.938	0.892	-0.052	0.008	-0.006	-0.011
Ptero04	150-168	11	10.45	0.821	0.826	0.006	10	8.22	0.906	0.812	-0.119	-0.003	0.021	-0.016
RBG29	124-136	9	7.51	0.653	0.805	0.215	6	5.90	0.813	0.796	-0.021	-0.014	-0.010	0.181
Parm02	192-198	6	5.00	0.415	0.411	0.003	5	4.20	0.375	0.489	0.235	0.006	-0.009	0.049
Paequ03	222-232	10	8.88	0.614	0.678	0.038	8	6.94	0.844	0.827	-0.020	0.019	-0.004	0.030

All *p*-values > 0.05.

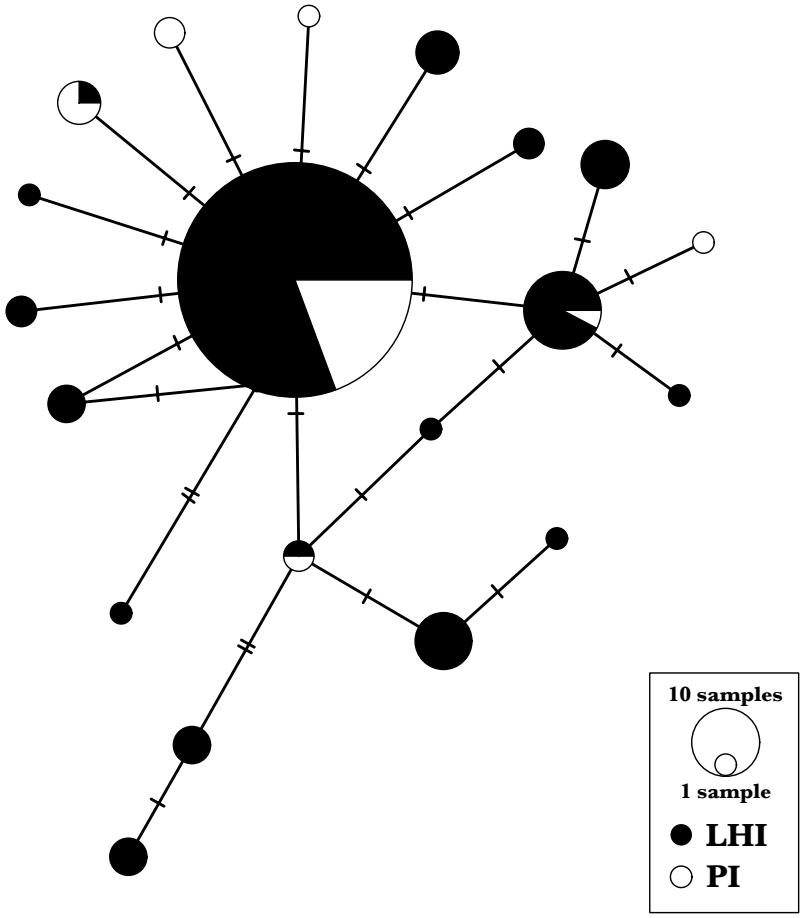

Figure 4 – 2 Haplotype network of providence petrel (*Pterodroma solandri*) mtDNA haplotypes based on the TCS algorithm. Haplotypes are represented by circles, where the size of each circle is proportional to the frequency of the corresponding haplotype. Lines on connecting branches represent mutations. Black: Lord Howe Island individuals. White: Phillip Island individuals.

Table 4 – 2 Summary statistics in *P. solandri* for the mitochondrial *Cytochrome b* gene and 14 nuclear introns. Pairwise population differentiation between Lord Howe Island and Phillip Island colonies, F_{st}, G_{st}, N_{st} and Φ_{st}, where Φ_{st} represents the direct analog of Wright's F_{st} for nucleotide sequence diversity (Excoffier *et al*., 1992)

Locus name	F_{st}	Φ_{st}	G_{st}	N_{st}
Cyt b	0.0105	0.1050	0.0061	0.0200
δ-cryst	0.0810	0.0810	0.0283	0.0810
Lipo2	0.0000	00000	0.0000	0.0000
Pema01	-0.0014	-0.0014	-0.0119	-0.0014
Pema05	0.0256	0.0256	0.0141	0.0256
Pema07	-0.0006	-0.0006	0.0135	0.0058
Pema10	0.0148	0.0148	0.0260	0.0148
Pema12	-0.0148	-0.0148	-0.0102	-0.0148
Pema13	-0.0129	-0.0129	-0.0208	-0.0129
Pema14	0.0203	0.0203	0.0130	0.0203
16214	-0.0002	-0.0002	-0.0117	-0.0002
20454	0.0166	0.0166	-0.0050	0.0166
22519	-0.0037	-0.0037	-0.0027	-0.0037
24206	-0.0221	-0.0221	-0.0197	-0.0221
24972	0.0170	0.0170	0.0178	0.0170

All *p*-values > 0.05.

.

Table 4 – 3 Pairwise differentiation matrix among *P. solandri* colonies. F_{st} among *Pterodroma solandri*. Lord Howe Island: Far Flats (FF), George's Bay (GB), Muttonbird Point (MBP), Mount Gower (MG). Phillip Island: Jacky Jacky (JJ). Above diagonal: pairwise differentiation matrix for mitochondrial DNA. Below diagonal: pairwise differentiation matrix for 10 microsatellites.

	JJ	**FF**	**MBP**	**GB**	**MG**
JJ	–	0.022	-0.003	0.042	0.022
FF	0.005	–	0.003	0.004	-0.017
MBP	-0.010	0.012	–	0.058	0.002
GB	-0.004	0.002	0.000	–	-0.002
MG	-0.003	0.003	0.004	-0.010	–

All p-values > 0.05.

Kinship coefficients (θ_{ij}) ranged from -0.28–0.69 and -0.27–0.36 within and between colonies, respectively. The analysis of variance of θ_{ij} showed no significant differences between 'within-colonies' and 'among-colonies' (pseudo-$F_{1,182} = 0.993$, $P = 0.424$). The clustering analysis, based on Calinski-Harabasz pseudo-F statistics, showed highest pseudo-F for $K>>2$ (Figure 4 – 3), which does not support Phillip Island and Lord Howe Island as genetically distinct colonies.

Bayesian clustering analysis and individual assignment

Evaluation of lnP(X/K), ΔK, and Q obtained with STRUCTURE supported $K = 4$, although genetic clusters did not reflect geographical localities. Each individual contained roughly equal coancestry from the four clusters (Supplementary Information SI. 4 – 5). The frequency-based and Bayesian assignment methods (Lord Howe Island vs. Phillip Island colonies) implemented in GENECLASS 2 showed 3 and 7 ($\alpha_{0.01}$), and 12 and 23 ($\alpha_{0.05}$) first-generation migrants, respectively (Supplementary Information SI. 4 – 6). Conversely, the two methods showed equivalent results with 59% of individuals correctly assigned (108 out of 183) with an average

probability of 54.28% at $\alpha_{0.05}$, and 53.39% at $\alpha_{0.01}$. This low confidence reflects the similarity between likelihoods of genotypes across populations.

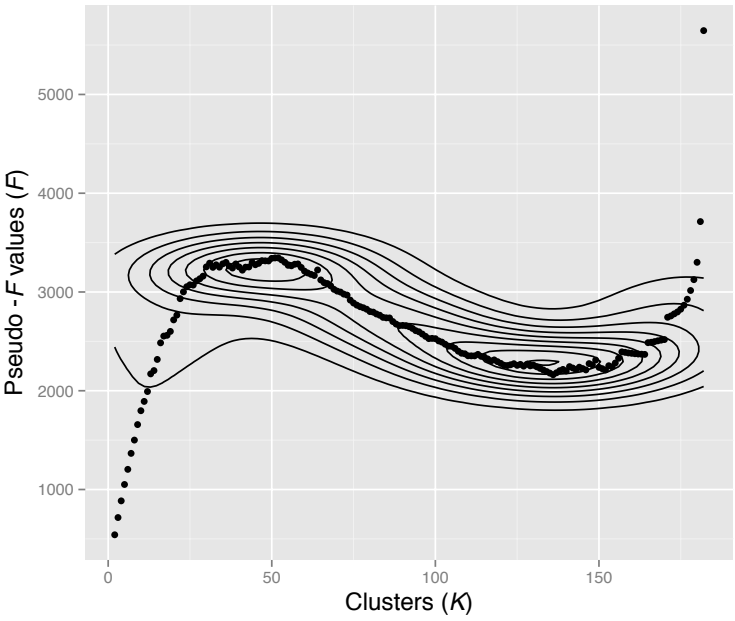

Figure 4 – 3 Calinski-Harabasz pseudo *F*-statistic density for kinship coefficients (K = 2–183). Highest density of pseudo-*F*-statistic values determine the most likely number of clusters among *P. solandri* individuals.

Estimation of divergence time

Implementations of the isolation-with-migration model using microsatellites, nuclear introns and mitochondrial loci resulted in unimodal posterior density curves of migration parameters, which were similar across the three runs. Migration rates were of 0.32 migrants/generation from the Phillip Island colony to the Lord Howe Island colony (0.24–0.49 90% HPD) (Figure 4 – 4a), and 8.6 migrants/generation from the Lord Howe Island colony to the Phillip Island colony (8.44-8.73 90% HPD) (Figure 4 – 4b). Divergence time estimates were also convergent across all analyses, corresponding to 88 years (56–200 90% HPD) (Figure 4 – 4c).

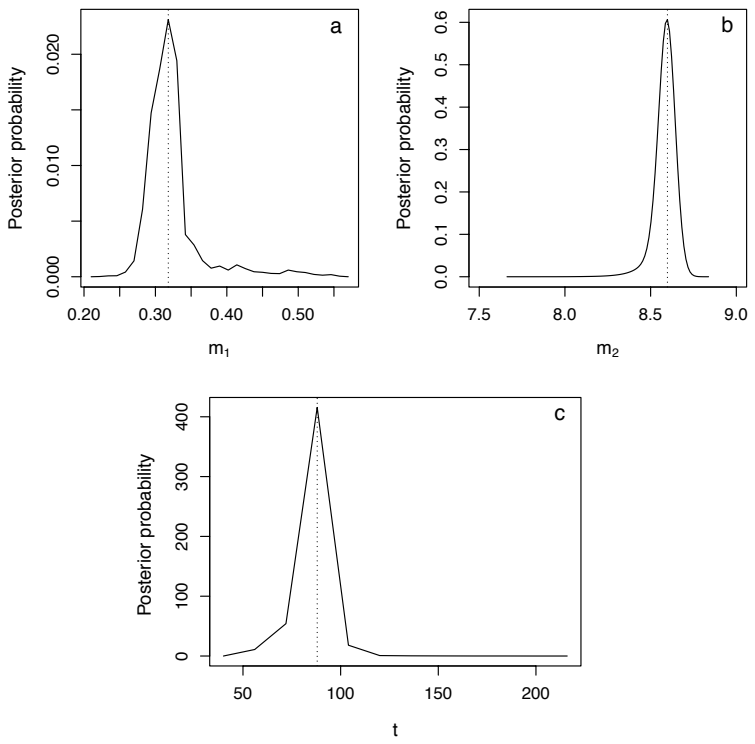

Figure 4 – 4 Population divergence genetic parameters. Marginal posterior probability distributions for the Isolation with Migration demographic parameters. a: migrants/generation from Lord Phillip Island to Lord Howe Island (m_1). b: migrants/generation from Lord Howe Island to Phillip Island (m_2). c: time of divergence (t, years).

Demographic history

Results from coalescent modelling of microsatellites using MsVar v0.4 and Msvar 1.3 both showed a strong signal for large population decrease in the Lord Howe Island colony (Figure 4 – 5a, 5b). Combining all simulations for all datasets, contemporary effective population size, N_0 (30 [0 – 1862], mean and 90% HPD) was three orders of magnitude smaller than the ancestral effective population size, N_1 (177,827 [5,888 – 4,265,795], mean and 90% HPD) (Figure 4 – 5b). All Bayes factors obtained with both methods were >>10 in favour of population decrease rather than increase. The time when the ancestral Lord Howe Island colony started to decrease (mean $\log_{10}(t_1) = 2.785$, exponential model) suggests a recent decrease in this colony (609 years, [20 – 10,000] mean and 90% HPD) (Figure 4 – 5c).

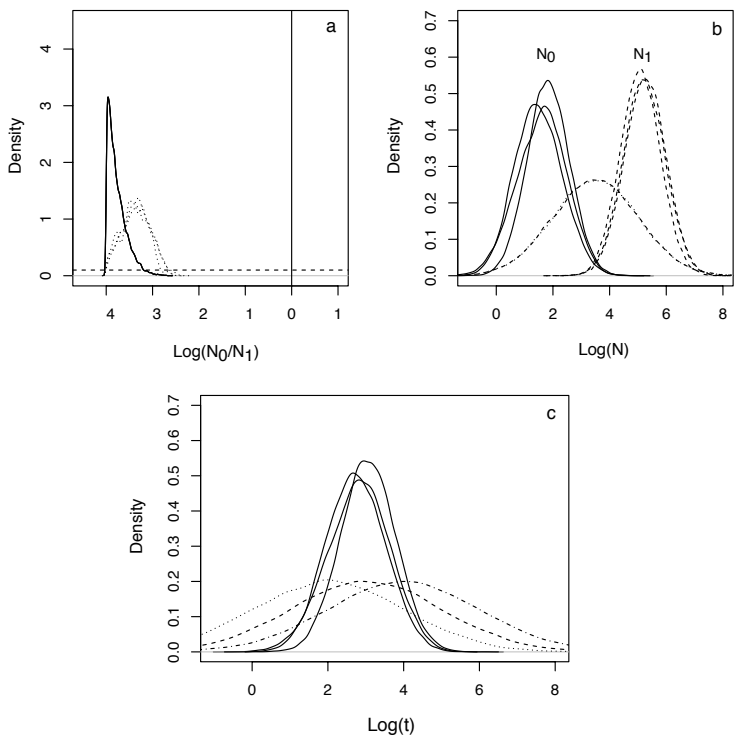

Figure 4 – 5 Population size change in *P. solandri* using coalescent modelling of microsatellite data under MsVar v0.4 and Msvar v1.3. a: posterior density distributions of the effective population size parameter Log(N_0/N_1) from MsVar v0.4 where 0 indicates population stability, <0 decline, and >0 expansion. Dotted curves represent the linear model and continuous curves represent the exponential model. The vertical solid line represents the expected value of Log(N_0/N_1) when the effective population size is stable. The straight horizontal dotted line represents the distribution of priors for comparison. b: posterior density distributions of the current (N_0, solid lines) and the ancestral (N_1, dotted lines) effective population size parameter Log(N) using MsVar v1.3 under the exponential model. c: posterior density distributions of the time parameter (Log(t), solid lines) since providence petrels started to decline on Lord Howe Island using MsVar v1.3 under the exponential model. The inferior dotted lines in fig. b and c represent the prior distributions of each parameter for comparison.

Discussion

I generated three genetic data sets consisting of DNA sequences from mitochondrial and 14 nuclear regions and genotypes from 10 microsatellite loci to investigate genetic connectivity and demographic history of providence petrel (*Pterodroma solandri*) colonies, an oceanic seabird IUCN uplisted as Vulnerable due to its restricted breeding range. High gene flow between the two remaining colonies of providence petrel (Lord Howe Island and Phillip Island) was evident despite individuals at the two colonies showing different time of return to nesting sites. In addition, time of divergence among colonies appears recent, suggesting recent colonization of Phillip Island by individuals from Lord Howe Island. These results suggest high plasticity in behaviour rather than adaptive divergence in providence petrels, and imply limited genetic risks surrounding the sustainability of the Phillip Island colony.

Contemporary genetic differentiation

The analyses conducted here on multiple datasets indicate high genetic connectivity between the two remaining populations of providence petrel (Lord Howe Island and Phillip Island). While low genetic divergence among populations could also reflect high historical connectivity between populations that are now isolated (Palsboll *et al*., 2010), I also investigated contemporary gene flow among populations. I compared the variation of kinship coefficients within and between providence petrel colonies (Lord Howe Island and Phillip Island), and showed that individuals coming from the same colony were as related genetically as individuals coming from different colonies; the best clustering of individuals was also independent of breeding locality. These results confirmed high current dispersal capacity of providence petrels, which suggests that species-wide genetic diversity is being maintained by natural dispersal between colonies.

Time of colonization

Maximum likelihood estimates obtained from the isolation-with-migration model showed that providence petrel colonies (Lord Howe Island and Phillip Island) became separated between 56 and 200 years ago. This suggests that individuals from Lord Howe Island were prospecting new habitats on Phillip Island after the extirpation of the Norfolk Island colony. These results indicate limited genetic risks surrounding the sustainability of the small Phillip Island colony of providence petrels. Indeed, as dispersal of prospectors is positively related to the presence of conspecifics (Serrano *et al*., 2004), one can expect additional gene flow from Lord Howe Island to Phillip Island in the near future. Conversely, the fact that the Phillip Island colony was only discovered in 1986 may be explained by the first explorations of this small island in the 1970s (Priddel *et al*., 2010). Analyses of ancient DNA samples from the Norfolk Island colony to assess its historical connectivity with Lord Howe Island are presented in Chapter 5.

Behavioral difference in timing of colony attendance

Despite providence petrel colonies being highly connected genetically, for the period of courtship and early incubation, Lord Howe Island individuals predominantly arrive at the colony during daylight, whereas Phillip Island individuals return to their breeding sites only after dusk. Numerous studies have illustrated the importance of behavioural plasticity as a fundamental trait of life history strategies in seabirds living in highly dynamic and variable environment (Falk *et al*., 2002; Paiva *et al*., 2009; Reed *et al*., 2009). Moreover, petrels have the capacity to use olfactory senses to find burrows at night, and this strategy is not exclusive to individuals showing nocturnal arrival at colonies (Bonadonna and Bretagnolle, 2002; Dell'Ariccia and Bonadonna, 2013). Individuals showing diurnal arrival are also able to use olfaction as the basic sensory input for homing at night, and use it if necessary (Dell'Ariccia and Bonadonna, 2013). These observations imply that all petrels are able to return to their burrows at night, and that individuals alter their behaviour to environmental conditions without

necessarily requiring genetic adaptation. Hence, it is likely that prospectors from Lord Howe Island have switched their behaviour on Phillip Island.

Earlier studies suggest that avoidance of predators is likely to be the main factor responsible for nocturnal colony arrival in small Procellariiformes (Watanuki, 1986; Warham, 1990; McNeil *et al*., 1993; Keitt *et al*., 2004). However, providence petrels from Phillip Island as well as other seabird species possess a nocturnal arrival behaviour even in the absence of diurnal predators (Keitt *et al*., 2004). Considering establishment of providence petrels on Phillip Island in the 1800s, this behaviour may also be a recent adaptation to the presence of hawks on the island at the time of European settlement (Medway, 2002b). These hawks apparently survived the 1st decade of European occupation of Norfolk Island (Medway, 2002b). Another explanation may be related to foraging, as has been observed for a number of seabird taxa (Baduini, 2002; Dias *et al*., 2012). For example, Cory's shearwaters (*Calonectris diomedea*) show intraspecific variation in colony arrival depending on the marine region and abundance of prey, and are high flexibility in their daily routines (Dias *et al*., 2012). However, unpublished logger data from Lord Howe Island individuals suggests foraging throughout the Coral and Tasman Seas during the breeding season (Carlile, per. obs.), such that it is difficult to imagine differences in foraging locations between Lord Howe and Phillip Island individuals.

Demographic history

Coalescent modelling of microsatellites indicated a past bottleneck in providence petrel. This significant decrease in population size is estimated to have occurred approximately 600 years ago. However, there is a broad uncertainty surrounding this date estimate. A survey of unconsolidated sediments on Lord Howe Island did not indicate human occupation of this island before the European era, beginning in 1788 (Anderson, 2003). However, various pieces of evidence ascribed to origins in Tonga or New-Zealand (e.g. pieces of wrecked canoes, adzes

made of local basalt and other wooden artefacts), as well as results of analyses of genetic variation in the Pacific rat (*Rattus exulans*) suggesting connectivity between Norfolk Island and New Zealand populations (Matisoo-Smith *et al*., 2001), constituted proof of Norfolk Island having been settled from New Zealand at about the thirteenth to fourteenth century (McCarthy, 1934; Anderson and White, 2001) Assuming that the Lord Howe colony was connected to the Norfolk Island colony (i.e. panmixia), the commencement of the bottleneck may be explained by the introduction Pacific rats or kiore (*Rattus exulans*) on Norfolk Island 600 yr B.P., as kiore is well known for having affected seabird species on other islands (Holdaway, 1999; Rayner *et al*., 2007). Polynesians may have also directly exploited the Norfolk population, as it has been seen elsewhere (Worthy, 1999; Holdaway and Jacomb, 2000; Boessenkool *et al*., 2009). Additionally or alternatively, given the arrival of Polynesians in New Zealand 700 yr B.P. (Wilmshurst and Higham, 2004), they may have also encountered Lord Howe Island at the same period. They may not have settled, which could explain lack of archaeological evidence, but allowed kiore (*Rattus exulans*) to colonise. Kiore may then have disappeared after the introduction of the ship rat (*Rattus rattus*) in 1918 (Hindwood, 1940). However, there is no evidence for Kiore ever having occupied Lord Howe Island.

Conservation implications

The local extirpation of providence petrels has had a severe impact on the terrestrial ecosystem of Norfolk Island, particularly through the deficiency of phosphorus leading to Norfolk Island pines (*Araucaria heterophylla*) being highly affected by the root-rotting fungus *Phellinus noxius* (Holdaway and Christian, 2010). To reduce the extinction risk of providence petrels and to provide key nutrients for the regeneration of threatened native forests and associated species, a plan to re-establish a colony of providence petrels on Norfolk Island using Lord Howe Island individuals has been proposed. Here I show that the small colony of providence petrels breeding on Phillip Island is genetically connected to the Lord Howe Island colony. These

results indicate limited risks surrounding the proposed translocation of Lord Howe Island individuals to re-establish a colony on Norfolk Island with respect to potential genetic novelty of the Phillip Island colony. In addition, as colonisation of Phillip Island has been recent, further gene flow will likely occur from Lord Howe Island to the Norfolk Island group, including the new translocated colony, reducing risks of inbreeding depression following translocation. While kiore is still present on Norfolk Island, this was not the proximate cause for providence petrel extinction from Norfolk Island. There is no obvious threat to other avian species on the island through this reintroduction.

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Supplementary Information SI. 4

Population genetic and behavioural variation of the two remaining colonies of providence petrel (*Pterodroma solandri*)

Anicee J. Lombal[1,2], Theodore J. Wenner, Nicholas Carlile, Jeremy J. Austin, Eric Woehler, David Priddel and Christopher P. Burridge. *Conservation Genetics*

[1]School of Biological Sciences, University of Tasmania, Hobart, TAS 7001, Australia. Phone: +61 3 6226 7653.

[2]Anicee.Lombal@utas.edu.au

The following sections are included in this Supplementary Information SI. 4:

SI. 4 – 1. Description of primers for 14 nuclear introns tested in *P. solandri*, fragment sizes, and successful PCR annealing temperatures (A_T)

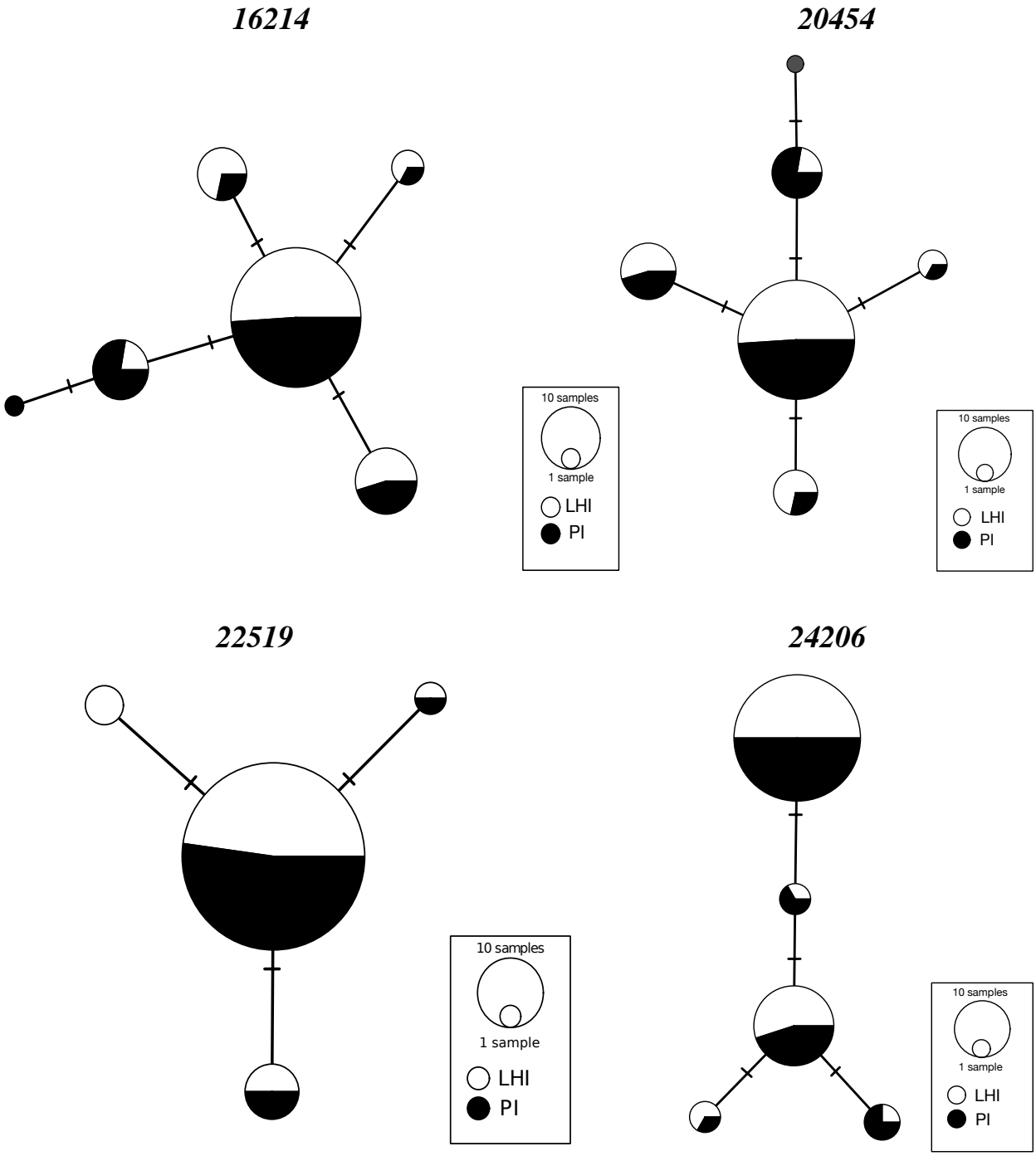
Locus nam	Source	Forward primer sequence	Reverse primer sequence	bp	A_T (°C)
δ-cryst	(Patterson *et al*., 2011)	5'-GCCCATCAGATGGAGCCAGTTC-3'	5'-CCAGGCGCTCAGAGTCACGGG-3'	194	55
Lipo2	(Patterson *et al*., 2011)	5'-AGTAAAACCTTTGTGGTGATCCAT-3'	5'-CATGGCAACATCCTTTCCCACCAGCTT-3'	262	55
Pema01	(Silva *et al*., 2011)	5'-ACACAGCCCTCCTTCAGAGA-3'	5'-TTAAGGCTGGACGATGCTCT-3'	603	55
Pema05	(Silva *et al*., 2011)	5'-GAATTCCTCCATCAGGGTGA-3'	5'-CATGCTCAAACCTGCAGAGA-3'	736	55
Pema07	(Silva *et al*., 2011)	5'-TGCCTCCAGTTTGCTAAGGT-3'	5'-AAAAGGAATTGCAGGTGTGG-3'	664	55
Pema10	(Silva *et al*., 2011)	5'-AAGCAGGGAGCTTGACAGAA-3'	5'-CAAACATCAGACAGCCTTGC-3'	656	60
Pema12	(Silva *et al*., 2011)	5'-GAACAGTGGGGCAACAATTT-3'	5'-TTCCCCAAGTCTTTTTGTGG-3'	661	55
Pema13	(Silva *et al*., 2011)	5'-TTCTTTCCTGTCCCCAGTTG-3'	5'-TGGGAAAAGCACCTATGGAA-3'	671	55
Pema14	(Silva *et al*., 2011)	5'-CCTAATCTTCCCTTTCACATGG-3'	5'-AGCAGTTAAGGGGTGCTGAA-3'	634	55
16214	(Backström *et al*., 2008)	5'-GCATACATCAGACCATCTCC-3'	5'-TCAACCATATCAGCCACAGC-3'	418	55
20454	(Backstrom *et al*., 2008)	5'-GTCCTGTGCCTTGTGTATGA-3'	5'-CATCTCACAGTATTCCAGGC-3'	433	50
22519	(Backstrom *et al*., 2008)	5'-TTTGAGACATATGAGCAGGC-3'	5'-TGTTTCTGAAGCTTCAAGTC-3'	635	60
24206	(Backstrom *et al*., 2008)	5'-TACCTGCAGCACCCAAGTTC-3'	5'-TTGGAAGTCCTTGAGTGATG-3'	463	50
24972	(Backstrom *et al*., 2008)	5'-CGTTCCACTAATATTTTCCG-3'	5'-GCTTCATCAGTGACTATGAC-3'	807	50

SI. 4 – 2. Parameters for the MCMC runs of program MSVAR 1.3

	Starting values (mean, variance)				Hyperpriors (α, σ, β, τ)				Run lengths		
	Log(N_0)	Log(N_1)	Log(μ)	Log(T)	Log(N_0)	Log(N_1)	Log(μ)	Log(T)	Steps	Thinning	Iterations
Run 1	3.5, 1.5	3.5, 1.5	-3.5, 1	2, 2	3.5, 1.5, 0.0, 0.5	3.5, 1.5, 0.0, 0.5	3.5, 1.5, 0.0, 0.5	2, 2, 0.0, 0.5	10^5	4.10^4	4.10^9
Run 2	3.5, 1.5	3.5, 1.5	-3.5, 1	3, 2	3.5, 1.5, 0.0, 0.5	3.5, 1.5, 0.0, 0.5	3.5, 1.5, 0.0, 0.5	3, 2, 0.0, 0.5	10^5	4.10^4	4.10^9
Run 3	3.5, 1.5	3.5, 1.5	-3.5, 1	4, 2	3.5, 1.5, 0.0, 0.5	3.5, 1.5, 0.0, 0.5	3.5, 1.5, 0.0, 0.5	4, 2, 0.0, 0.5	10^5	4.10^4	4.10^9

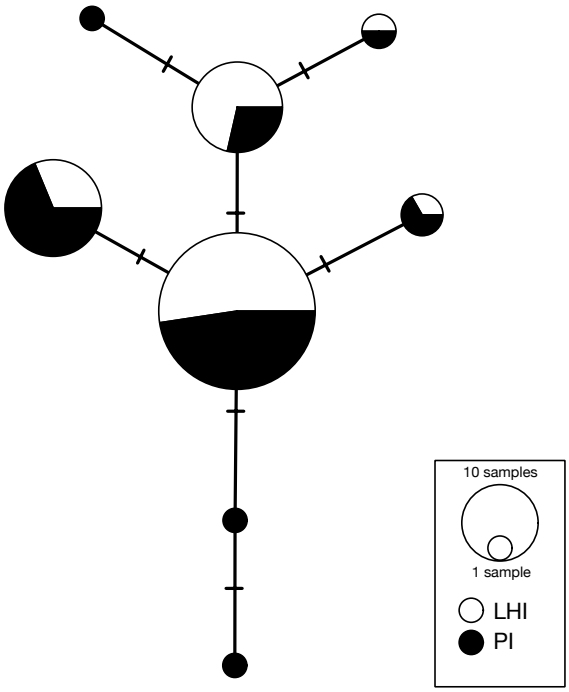
SI. 4 – 3 Characterization of genetic diversity and variation from neutral expectations in *P. solandri* for the mitochondrial *Cytochrome b* gene and 14 nuclear introns. Genetic variation within the two colonies: number of haplotypes (A), haplotypic diversity (*h*), haplotype ratios (X_H), nucleotide diversity (π), and nucleotide diversity ratios (π_R). Deviation from neutral expectations: Tajima's *D* test (Tajima, 1989) and Fu and Li's *D** test (Fu and Li, 1993).

			P. solandri Lord Howe Island								*P. solandri* Phillip Island							
Locus	Length (bp)	Genbank accession #	n	A	h	X_H	π	π_R	*D*	*D**	*n*	A	h	X_H	π	π_R	*D*	*D**
Cyt *b*	872	KX123006	151	17	0.561	0.850	0.0117	1.864	-1.987*	-2.920*	32	7	0.482	0.350	0.0069	0.536	-1.670	-1.150
δ-cryst	194	KX123189	20	14	0.884	0.778	0.0109	0.727	0.664	0.821	20	12	0.870	0.667	0.0151	1.375	-2.030*	0.820
Lipo2	262	KX123269	20	1	0.000	0.500	0.0000	-	0.000	0.000	20	2	0.100	1.000	0.0051	-	-1.164	-1.540
Pema01	603	KX122606	20	6	0.756	0.778	0.0189	1.105	-0.505	-0.456	20	9	0.754	0.889	0.0179	0.948	0.944	-0.232
Pema05	736	KX122446	20	3	0.097	0.500	0.0013	0.333	-0.836	0.564	20	3	0.188	0.750	0.0040	3.000	-1.645	-2.167*
Pema07	664	KX122526	20	8	0.723	0.727	0.0241	0.854	-0.077	1.256	20	7	0.647	0.636	0.0238	1.170	-0.110	0.512
Pema10	656	KX122686	20	5	0.669	0.833	0.0126	0.722	-0.291	-0.038	20	5	0.781	0.833	0.0174	1.385	1.351	0.916
Pema12	661	KX122766	20	5	0.444	0.714	0.0126	0.863	-0.271	-0.038	20	6	0.565	0.857	0.0146	1.158	-0.455	-1.662
Pema13	671	KX122926	20	5	0.686	0.833	0.0159	1.103	0.324	-0.038	20	5	0.658	0.833	0.0144	0.906	0.070	-0.238
Pema14	634	KX122846	20	6	0.633	0.750	0.0124	0.809	-0.850	-1.660	20	7	0.677	0.875	0.0153	1.235	-1.140	-1.720
16214	418	KX122126	20	8	0.742	0.800	0.0339	0.741	-1.533	-3.110*	20	8	0.753	0.800	0.0458	1.350	-0.790	-0.732
20454	433	KX122046	20	5	0.581	0.833	0.0157	0.908	-0.660	1.027	20	6	0.605	1.000	0.0173	1.102	-0.921	-0.736
22519	635	KX122206	20	4	0.315	1.000	0.0053	1.740	-1.146	-0.350	20	3	0.188	0.750	0.0030	0.574	-1.112	-0.828
24206	463	KX122286	20	5	0.543	1.000	0.0236	0.975	0.392	-0.038	20	5	0.564	1.000	0.0243	1.026	0.464	-0.038
24972	807	KX122366	20	13	0.877	1.000	0.0265	1.261	0.035	0.107	20	8	0.744	0.615	0.0210	0.793	-0.278	1.311



SI. 4 – 4 Haplotype network of providence petrel (*Pterodroma solandri*) nuclear introns haplotypes based on the TCS algorithm. Haplotypes are represented by circles, where the size of each circle is proportional to the frequency of the corresponding haplotype. Lines on connecting branches represent one single mutation. White: Lord Howe Island individuals; dark grey: Phillip Island individuals. Figure continues over subsequent pages.

Pema14



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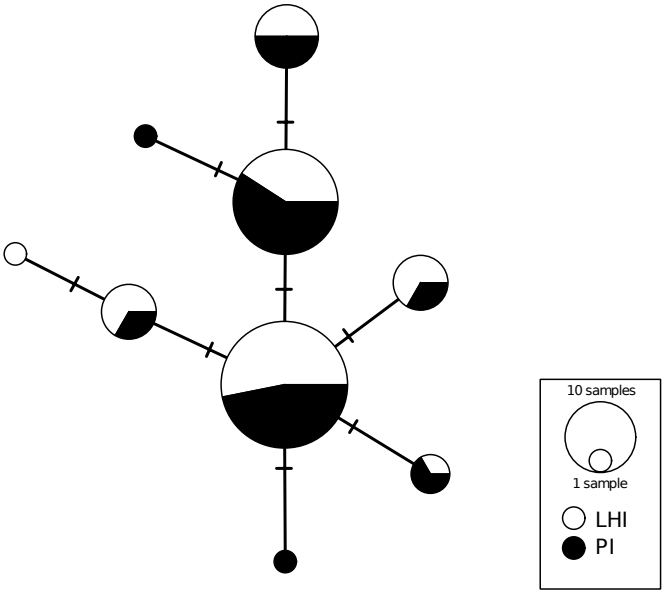

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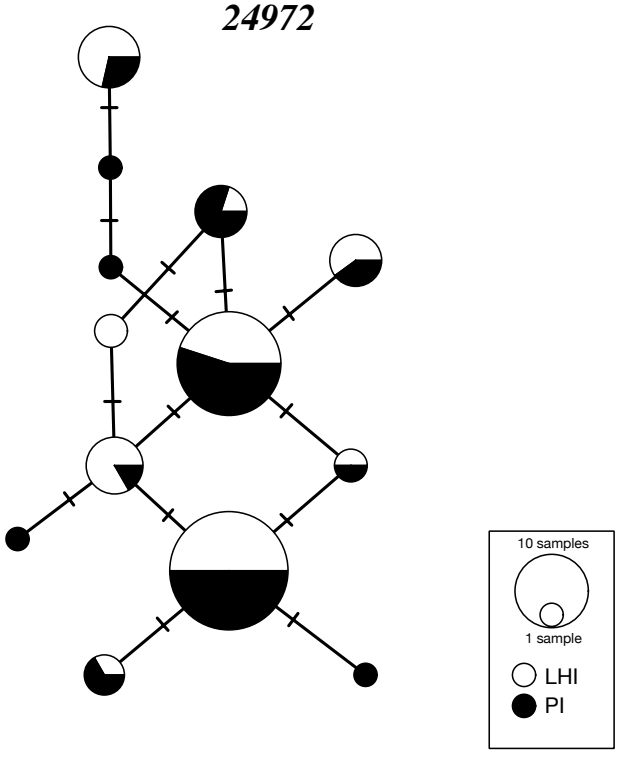
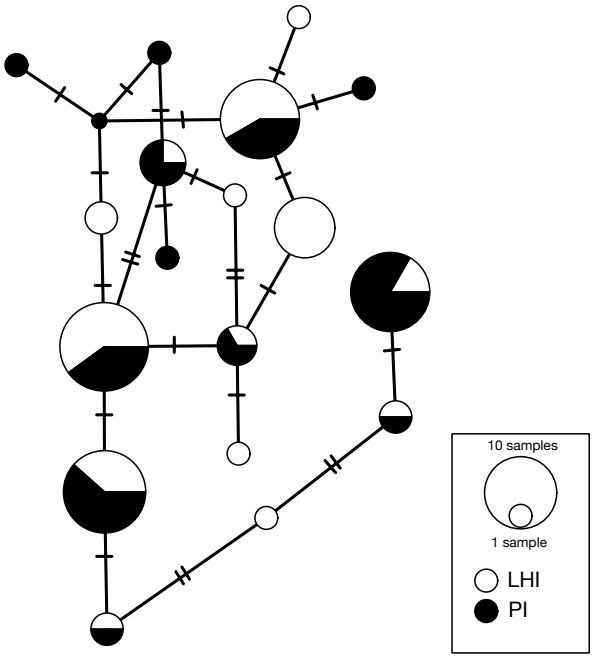
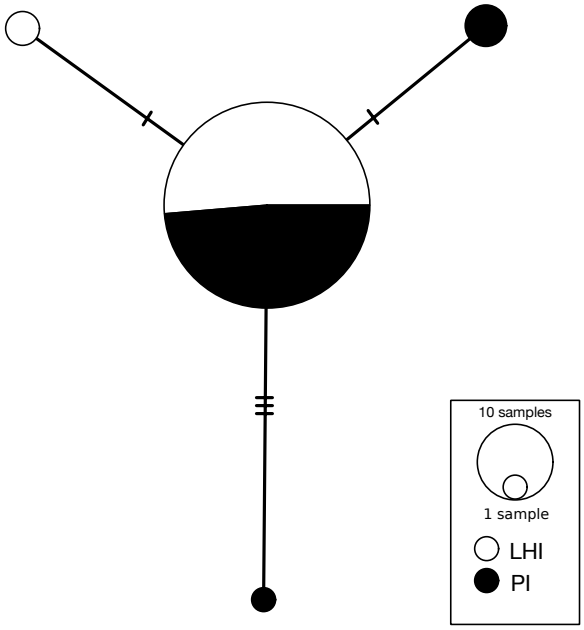


d-cryst



Pema5



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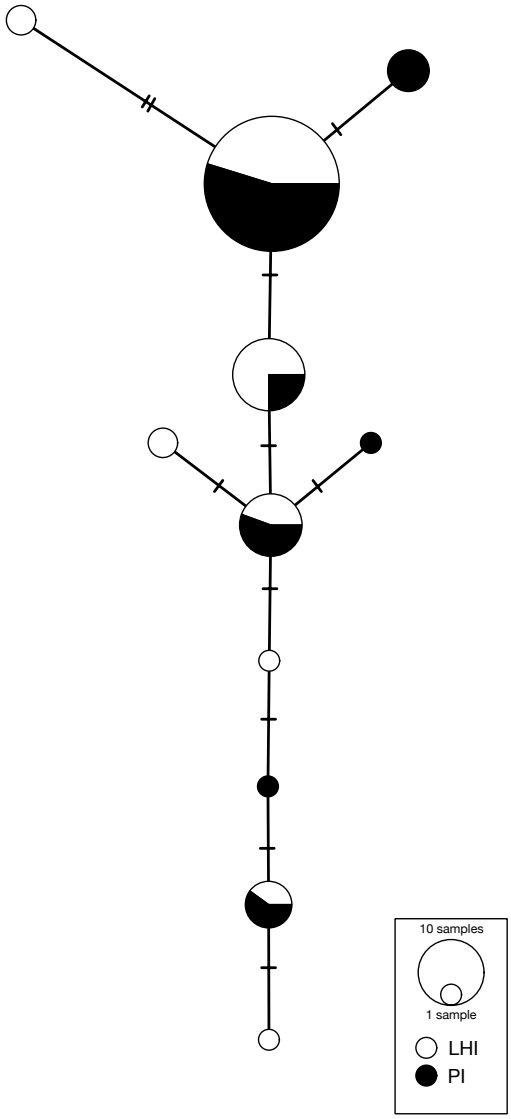
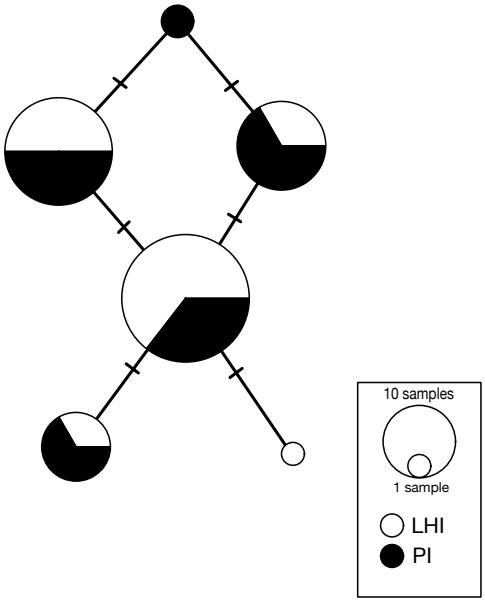


Pema10



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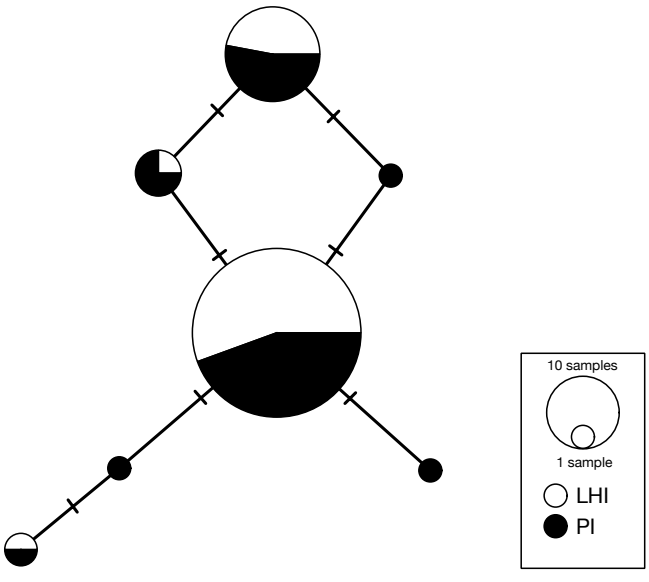
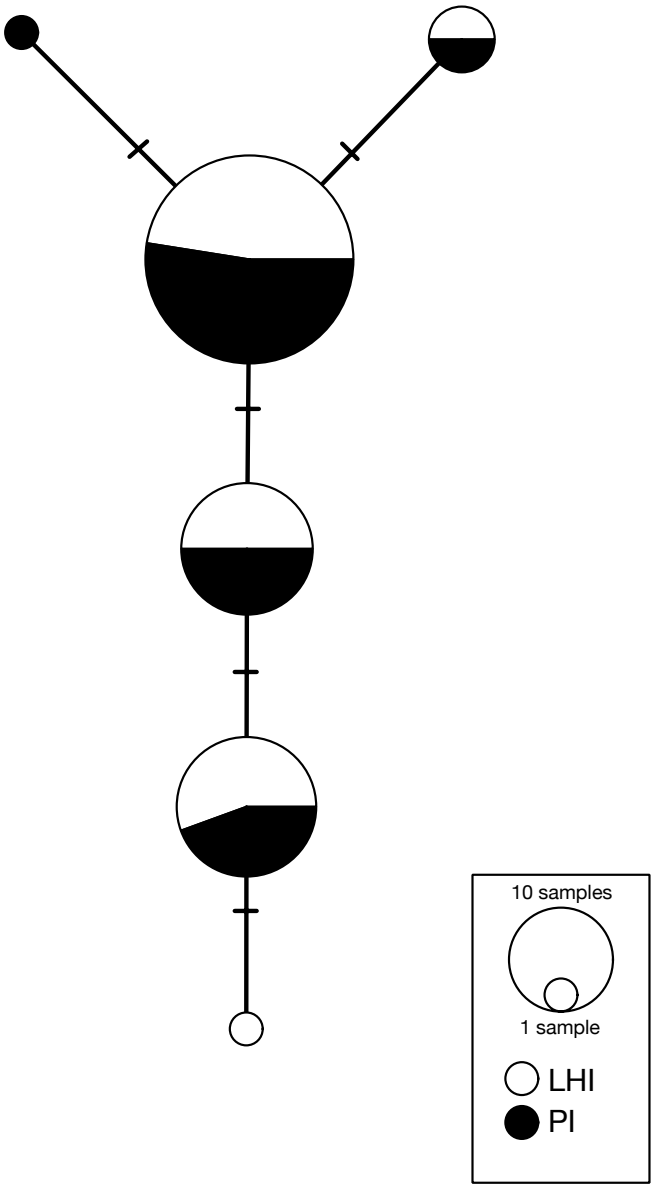


Pema13

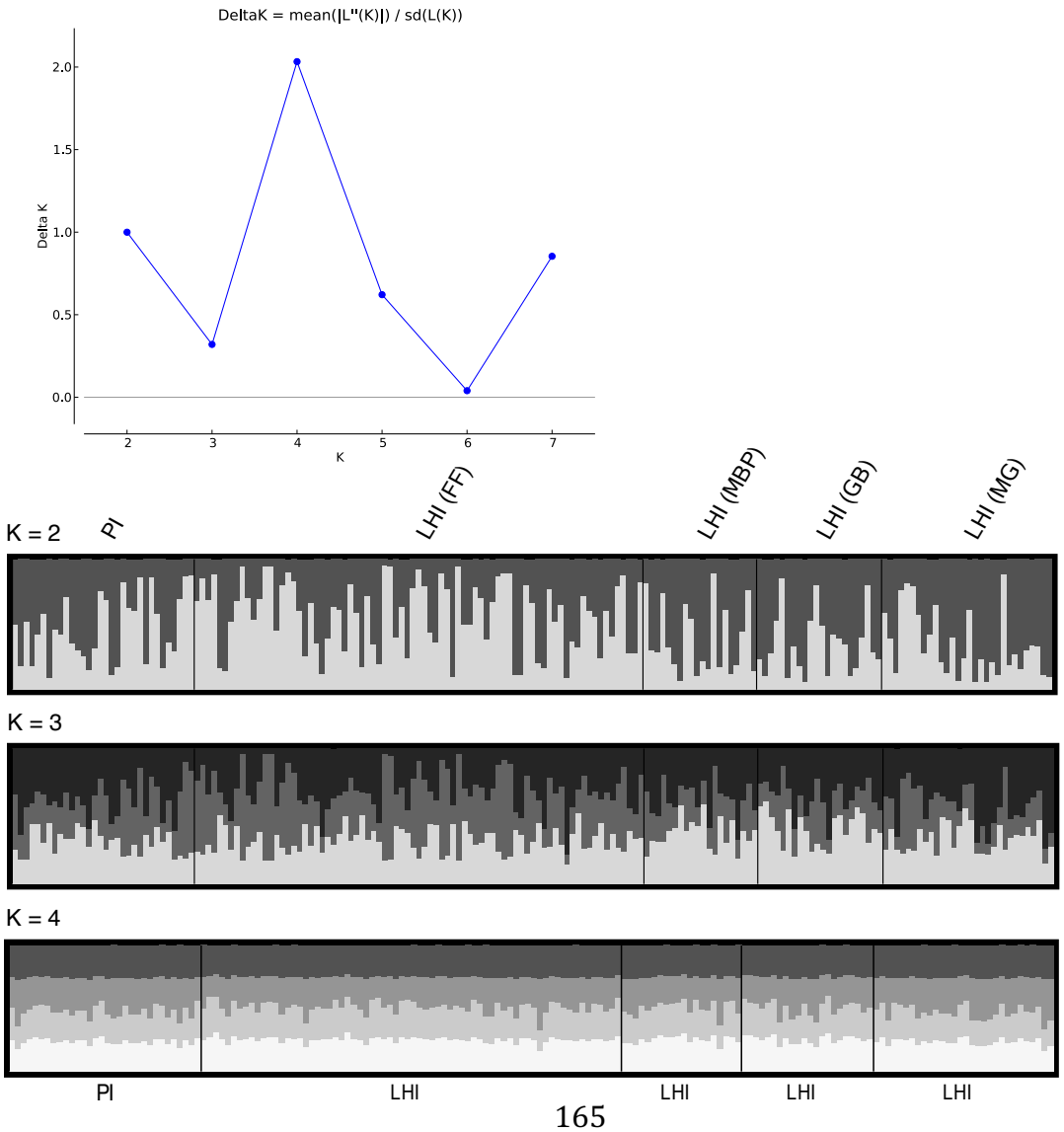


SI. 4 – 5 Population structure among providence petrel sampling localities inferred from microsatellite Bayesian clustering. A) the Evanno table output obtained with STRUCTURE Harvester (K = 1 – 8). B) the proportion of ancestry assigned to each of the two clusters plotted by individual for K = 2 – 4.

A The Evanno table output obtained with STRUCTURE Harvester in *P. solandri* for 10 microsatellites

K	Reps	MeanLnP(K)	Stdev Ln(P(K)	Ln'(K)	Ln''(K)	ΔK
1	20	-5921.960000	0.201108	-	-	
2	20	-6402.420000	139.518297	-480.460000	139.470000	0.999654
3	20	-7022.350000	222.369948	-619.930000	71.170000	0.320052
4	**20**	**-7713.450000**	**529.017131**	**-691.100000**	**1075.340000**	**2.032713**
5	20	-7329.210000	349.220677	384.240000	217.000000	0.621384
6	20	-7161.970000	237.337903	167.240000	9.440000	0.039775
7	20	-6985.290000	157.983406	176.680000	134.900000	0.853887
8	20	-6943.510000	93.318671	41.780000	-	-

B



SI. 4 – 6. First-generation migrants obtained with GENECLASS 2 using the frequency-based (Paetkau *et al.*, 2004) and the Bayesian assignment (Rannala and Mountain, 1997) methods. PI: Phillip Island; LHI: Lord Howe Island.

	$\alpha = 0.05$				$\alpha = 0.01$			
	Frequency		Bayesian		Frequency		Bayesian	
Sampled from	$-\text{Log}_{10}(L_o/L_{max})$	P	$-\text{Log}_{10}(L_o/L_{max})$	P	$-\text{Log}_{10}(L_o/L_{max})$	P	$-\text{Log}_{10}(L_o/L_{max})$	P
PI	2.258	0.0048	5.213	0.0011	2.258	0.0048	5.213	0.0011
PI	-	-	-	-	-	-	2.786	0.0287
PI	-	-	-	-	1.466	0.0279	3.043	0.0245
PI	-	-	-	-	1.322	0.0390	2.241	0.0475
PI	-	-	-	-	-	-	2.327	0.0447
PI	-	-	-	-	1.651	0.0199	3.172	0.0204
LHI	-	-	-	-	1.056	0.0350	1.063	0.0297
LHI	-	-	-	-	-	-	0.867	0.0387
LHI	-	-	1.846	0.0091	-	-	1.846	0.0091
LHI	-	-	-	-	0.981	0.0398	0.993	0.0342
LHI	-	-	-	-	-	-	0.714	0.0483
LHI	-	-	-	-	1.531	0.0112	1.443	0.0185
LHI	-	-	2.210	0.0064	0.905	0.0475	2.210	0.0064
LHI	-	-	-	-	-	-	0.743	0.0473
LHI	1.623	0.0088	4.445	0.0001	1.623	0.0088	4.445	0.0001
LHI	-	-	-	-	-	-	0.970	0.0337
LHI	-	-	-	-	1.074	0.0317	1.028	0.0314
LHI	-	-	2.257	0.0060	-	-	2.257	0.0060
LHI	-	-	2.968	0.0020	-	-	2.968	0.0020
LHI	-	-	1.784	0.0010	1.196	0.0267	1.784	0.0010
LHI	1.659	0.0080	-	-	1.659	0.0080	-	-
LHI	-	-	-	-	-	-	0.847	0.0403
LHI	-	-	-	-	-	-	1.599	0.0150
LHI	-	-	-	-	-	-	0.979	0.0339

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Chapter 5:

Ancient DNA reveals population extinction despite genetic connectivity in the providence petrel (*Pterodroma solandri*)

Ancient DNA reveals population extinction despite genetic connectivity in the providence petrel (*Pterodroma solandri*)

Anicee J. Lombal[1,6], Kieren Mitchell[2,7], Alexander T. Salis[2,8], Alan Tennyson[3,9], Lara Sheperd[3,10], Trevor Worthy[4,11], Eric J. Woehler[5,12], Jeremy J. Austin[2,13] and Christopher P. Burridge[1,14].

[1]Discipline of Biological Sciences, School of Natural Sciences, University of Tasmania, Hobart, TAS 7001, Australia.

[2]Australian Centre for Ancient DNA, School of Biological Sciences, University of Adelaide, Adelaide, SA 5005, Australia.

[3]Museum of New Zealand Te Papa Tongarewa 55 Cable Street, Te Aro, Wellington 6011, New Zealand.

[4]Biological Sciences, Flinders University, Adelaide, SA 5001, Australia.

[5]Institute for Marine and Antarctic Studies, University of Tasmania, Hobart, TAS 7053, Australia.

[6]Anicee.Lombal@utas.edu.au

[7]Kieren.Mitchell@adelaide.edu.au

[8]Alexander.Salis@adelaide.edu.au

[9]AlanT@tepapa.govt.nz

[10]Lara.Sheperd@tepapa.govt.nz

[11]Trevor.Worthy@flinders.edu.au

[12]Eric.Woehler@utas.edu.au

[13]Jeremy.Austin@adelaide.edu.au

[14]Chris.Burridge@utas.edu.au

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Abstract

Connectivity among populations is widely accepted as beneficial for species persistence from both genetic and demographic perspectives, and its re-establishment following population fragmentation is strongly advocated to attenuate population decline. However, the benefits of connectivity for offsetting negative processes operating on populations in the wild is difficult to assess, as it is challenging to determine the level of connectivity experienced by now-extinct populations. Here I used heterochronous sampling for one mitochondrial DNA marker (Cytochrome b) in a potentially high gene flow species – the providence petrel (*Pterodroma solandri*) – to assess whether population extinction on Norfolk Island occurred in the presence of connectivity. I sampled providence petrels representing contemporary (Lord Howe and Phillip Island) and extinct (Norfolk Island) populations, the latter comprising ~1,000,000 breeding pairs prior to extirpation by humans following settlement circa 1800. The majority of subfossil Norfolk Island individuals exhibited the most common mitochondrial haplotype from Lord Howe Island, consistent with high genetic connectivity between the two populations. These results reveal that even very large seabird populations can be rapidly extirpated by humans despite genetic connectivity with unaffected populations, which has significant conservation implications for predicting the resilience of other species.

Introduction

Connectivity among populations is considered beneficial for population persistence, by reducing levels of inbreeding, increasing genetic diversity, and providing demographic and fitness benefits (Frankham, 1996). For instance, Allendorf (1991) found that the introduction of a few grizzly bears (*Ursus arctos* ssp.) per generation significantly reduced the decline of genetic variation in the isolated Rocky Mountains population. Similarly, Spielman and Frankham (1992) documented significant increases in reproductive fitness in ten replicate inbred lines of *Drosophila melanogaster* that received only one migrant per generation. Concordantly, the majority of population extinctions observed in nature involve species with low dispersal ability (e.g. insular populations, Frankham, 2008), although population extinctions have still been observed in species with high dispersal potential, such as seabirds, suggesting that the ability of connectivity to attenuate decline has limits. However, it has typically been challenging to quantify the actual level of connectivity between populations of such species after population extinction.

Ancient DNA has revolutionized the field of conservation genetics as specific conservation issues may be informed by the genetic analysis of historic populations (Hofreiter *et al*., 2001; Leonard, 2008; Orlando and Cooper, 2014). Most commonly this involves the reconstitution of past demographic trajectories and testing for coincidence of decline with putative causes of extinction, such as the arrival of humans or changes in climate (e.g. Wilmshurst *et al*., 2014; Brüniche-Olsen *et al*., 2018). Alternatively, aDNA studies may document cryptic loss of genetic and species diversity (e.g. Calvignac *et al*., 2008; Ramírez *et al*., 2013). However, the potential of aDNA studies to quantify connectivity among populations prior to extinctions, and to assess the value of immigration to offset the processes that have driven these extinctions, has been less commonly realised.

The providence petrel *Pterodroma solandri* is classified as vulnerable under both the *IUCN Red List of Threatened Animals* (Criteria D2) and the *New South Wales Threatened Species Conservation Act 1995* due to its restricted breeding range. The only significant contemporary breeding locality is Lord Howe Island (~32,000 breeding pairs (Bester, 2003), a small island located 600 km off the east coast of Australia (Figure 5 – 1). Around 1,000,000 pairs bred on Norfolk Island, located 900 km northeast of Lord Howe Island (Figure 5 –1), before becoming extirpated following settlement by Europeans in the late 18th century (Medway, 2002a). The main contributors to population decline were direct exploitation by humans (~1,000,000 adults and young harvested in the four breeding seasons from 1790 to 1793 alone, Medway, 2002a) along with predation and disruption of breeding by introduced mammals (pigs, rats; Schodde *et al*., 1983). *Pterodroma solandri* was considered extinct within the Norfolk Island group until 1986 when a small population (~20 breeding pairs) was discovered on Phillip Island, 7 km south of Norfolk Island (Hermes *et al*., 1986) (Figure 5 –1). A comprehensive study of the genetic distinctiveness between the two extant breeding colonies using three genetic data sets – DNA sequences from mitochondrial and 14 nuclear regions, and genotypes from 10 microsatellite loci – showed high connectivity between colonies (Lombal *et al*., 2016; see Chapter 4). However, it is not known whether the Norfolk colony went extinct while genetically connected with other colonies. Furthermore, it is not known whether this extinction may reflect cryptic loss of species diversity (Ramakrishnan and Hadly, 2009).

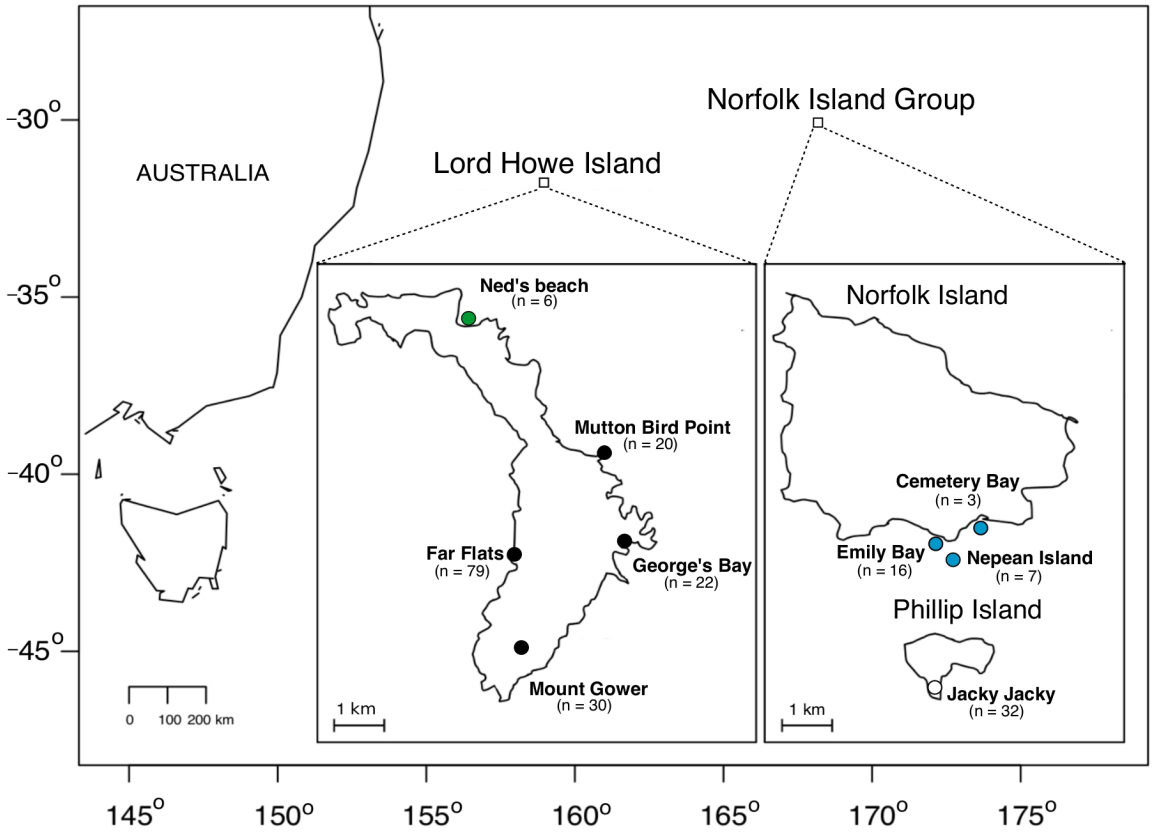

Figure 5 – 1 Locations of *Pterodroma* samples. Black: Modern *P. solandri* (Lombal *et al.*, 2017) collected on Lord Howe Island (n=151); white: modern *P. solandri* samples collected on Phillip Island (n=32). Total number of modern samples successfully sequenced = 176. Green: ancient *Pterodroma* sp. collected on Lord Howe Island (n=6); blue: ancient *Pterodroma* sp. collected on Norfolk Island (n=26).

While there is no morphological evidence justifying taxonomic separation between the extinct Norfolk Island colony and the two extant colonies of providence petrels, they differed behaviorally, and such differences can constitute barriers to genetic exchange in seabirds (e.g. allochrony; Smith and Friesen, 2007). Lord Howe Island individuals predominantly return to the colony during daylight (Bester *et al*., 2002; Medway, 2002b), while Phillip Island individuals return only after dusk (A. Lombal & A. Tennyson pers. obs.). Nocturnal return was similarly reported for the Norfolk Island population, and may relate to the presence of diurnal aerial predators — brown goshawks *Accipiter fasciatus* — present at the time of European settlement on Norfolk Island (Medway, 2002b). Information on past connectivity between providence petrel colonies may therefore inform whether the nocturnal behavior observed for Phillip Island individuals reflects an ancestral adaptation to diurnal predators on Norfolk Island, or a recent adaptation to new environmental conditions.

There are suggestions that other species of *Pterodroma* may have historically bred on Norfolk Island, with small fragmentary bone material common in middens particularly difficult to identify (Holdaway and Anderson, 2001). *Pterodroma solandri* are distinguishable from other *Pterodroma* based on their size, and Meredith (Meredith, 1985) recorded an unnamed *Pterodroma* that was smaller in size than *P. solandri* and this is now thought to be *P. neglecta* (see (Holdaway and Anderson, 2001)). The dimensions and colour pattern of a small *Pterodroma* painted at the time of first European settlement (Hindwood, 1965) fit those of *Pt. pycrofti* (Holdaway and Anderson, 2001), although Whitley (Whitley, 1938) described the bird represented by the painting as a new species, *Cookilaria hindwoodi*.

In this study, I used ancient DNA methods to compare mitochondrial Cytochrome *b* sequences from Norfolk Island Holocene subfossil to those of modern petrels. My main goal was to quantify whether the Norfolk Island providence petrel colony declined in the presence of connectivity with other populations, which is essential to assess the ability of connectivity to attenuate processes that have driven extinctions. My study also tested whether genetic distinctiveness was lost during the Norfolk extinction, and whether the nocturnal behavior of contemporary Phillip Island individuals reflects a genetic legacy of past connection to the Norfolk population. Lastly, I examined the identity of the small *Pterodroma* bones from Norfolk Island that may represent a species that does not presently breed there (another extinction event).

Materials and Methods

Sampling, ancient DNA extractions and high-throughput Sequencing

Subfossil specimens (n = 26) representing part of the Richard Holdaway and Atholl Anderson paleontological excavations on Norfolk Island between 1995 and 1997 (Holdaway and Anderson, 2001) were sampled from the museum of New Zealand Te Papa Tongarewa (n = 16), and the Australian National Wildlife Collections (ANWC n = 10; see details in Table 5 – 1). Among those specimens, 10 were provisionally morphologically identified as *Pterodroma* sp., 10 were identified as *P. solandri* and six were identified as *P. pycrofti* (Table 5 – 1). Additional subfossil specimens collected on Lord Howe Island and identified as *P. solandri* were obtained from ANWC and analysed in the present study (n = 6, see details in Table 5 – 1).

DNA extractions and high-throughput sequencing were employed to sequence all specimens (n = 32). The Te Papa samples (n = 16) were processed in the dedicated ancient DNA facilities

at the Australian Centre for Ancient DNA (ACAD) at the University of Adelaide while the ANWC samples (n = 16) were processed in another clean-room at the University of Adelaide. Strict protocols were followed and a number of precautions taken to minimize contamination of samples with exogenous DNA (Cooper, 2000). Potential surface contamination on the samples was reduced by UV irradiation for 15 min each side followed by abrading the exterior surface (c. 1 mm) using a Dremel tool and a disposable carborundum disk. The sample was then pulverized with a metallic mallet and approximately 100 mg of powder used for extraction. DNA was extracted using an in-house silica-based extraction protocol adapted from Dabney *et al*. (2013). The powder was digested first in 1 mL 0.5 M EDTA for 60 min, followed by an overnight incubation in 1 mL fresh 0.5 M EDTA containing 600 μg proteinase K at 55°C. The samples were centrifuged and the supernatant mixed with 13 mL of a modified PB buffer (Qiagen) containing 0.0005% Tween-20 and 0.09M Sodium Acetate and bound to silicon dioxide particles, which were then washed twice with 80% ethanol. The DNA was eluted from silica particles with 100 μL TE buffer. Extraction blank (negative) controls were processed alongside the samples.

Double-stranded Illumina libraries were built from 25 µL of DNA extract using partial uracil-DNA glycosylase (UDG) treatment (Rohland *et al*., 2015) with truncated Illumina adapters with unique dual 7-mer internal barcodes to allow identification and exclusion of any downstream contamination. A short round of PCR using PCR primers complementary to the adapter sequences was performed to increase the total amount of DNA. Cycle number was determined via rtPCR and split into 8 separate PCRs per library to minimize PCR bias and maintain complexity. Each PCR of 25 μL contained 1× HiFi buffer, 2.5 mM MgSO4, 1 mM dNTPs, 0.5 mM each primer, 0.1 U Platinum Taq Hi-Fi polymerase and 2 µL DNA. The cycling conditions were 94 °C for 12 min, 13–23 cycles of 94 °C for 30 s, 60 °C for 30 s, and

68 °C for 40 s, followed by and 68 °C for 10 min. PCR replicates were pooled and purified using AxyPrep™ magnetic beads (Axygen™). DNA was eluted in 30 μL EB buffer and quantified with a Qubit fluorometer (Thermo Fisher).

Commercially synthesised biotinylated 80-mer RNA baits (Arbor Biosciences, MI, USA) were used to enrich the libraries for avian mitochondrial DNA (Mitchell *et al*., 2014). DNA-RNA hybridisation enrichment was performed according to manufacturer's recommendations (MYbaits protocol, v1 for the ANWC samples and v3 for the Te Papa samples) with the exception that we used 1.25 µL of baits per reaction and changed the incubation step to: 55 °C for 15 hr followed by 50 °C for 16 hr (for Te Papa samples), or 3 h at 60 °C, 12 h at 55 °C, 12 h at 50 °C, then 17 h at 55 °C (for ANWC samples). The beads were washed three times with 0.1x SSC and 0.1% SDS solution (5 min, 50 °C) for Te Papa samples, or three times with MYbaits wash buffer v1 (5 min, 55 °C) for ANWC samples. Full-length Illumina sequencing adapters were then added to the enriched libraries via a final round of "off-bead" PCR split into five replicate PCRs (25 μL) containing 1× Gold PCR buffer, 2.5 mM $MgCl_2$, 1 mM dNTPs, 0.5 mM each primer (IS4 & indexing primer; (Meyer and Kircher, 2010)) and 0.1 U AmpliTaq Gold. Cycling conditions were as follows: 94 °C for 12 min, 15 cycles of 94 °C for 30 s, 60 °C for 30 s, 72 °C for 40 s, followed by 72 °C for 10 min. PCR replicates were pooled and purified as before but eluted in 30 μL H_2O and quantified on a TapeStation (Agilent Technologies). Enriched petrel libraries were pooled, diluted to 2 nM, and run on an Illumina NextSeq 500 (2 x 150 paired end for ANWC samples, 2 x 75 paired end for Te Papa samples).

Table 5 – 1 Ancient samples obtained from bones at the Museum of New Zealand, Te Papa, and the Australian National Wildlife Collections (ANWC), representing Lord Howe Island (LHI) and Norfolk Island (NI) specimens, including morphological identification. * = successfully sequenced

Accession#	ACAD#	Locality		Morphological ID	GenBank Accession #
Australian National Wildlife Collections (ANWC)					
ANWC P00123	17912	LHI	Ned's Beach	*Pterodroma solandri*	
ANWC P00124	17913	LHI	Ned's Beach*	*Pterodroma solandri*	MH828436
ANWC P00125	17914	LHI	Ned's Beach*	*Pterodroma solandri*	MH828437
ANWC P00126	17915	NI	Cemetery Bay*	*Pterodroma* sp.	MH828438
ANWC P00127	17916	NI	Cemetery Bay	*Pterodroma* sp.	
ANWC P00128	17917	NI	Cemetery Bay*	*Pterodroma* sp.	MH828439
ANWC P00129	17918	LHI	Ned's Beach*	*Pterodroma solandri*	MH828440
ANWC P00130	17919	LHI	Ned's Beach*	*Pterodroma solandri*	MH828441
ANWC P00131	17920	LHI	Ned's Beach*	*Pterodroma solandri*	MH828442
ANWC P00132	17921	NI	Nepean Island	*Pterodroma* sp.	
ANWC P00133	17922	NI	Nepean Island*	*Pterodroma* sp.	MH828443
ANWC P00134	17923	NI	Nepean Island*	*Pterodroma* sp.	MH828444
ANWC P00135	17924	NI	Nepean Island	*Pterodroma* sp.	
ANWC P00136	17925	NI	Nepean Island	*Pterodroma* sp.	
ANWC P00137	17926	NI	Nepean Island*	*Pterodroma* sp.	MH828445
ANWC P00138	17927	NI	Nepean Island	*Pterodroma* sp.	
Te Papa Museum					
S45698.1	20192	NI	Emily Bay*	*Pterodroma solandri*	MH828424
S45700.2	20193	NI	Emily Bay*	*Pterodroma solandri*	MH828425
S45702.3	20194	NI	Emily Bay*	*Pterodroma solandri*	MH828426
S45703.2	20195	NI	Emily Bay*	*Pterodroma solandri*	MH828427
S45704.5	20196	NI	Emily Bay*	*Pterodroma solandri*	MH828428
S45708	20197	NI	Emily Bay	*Pterodroma solandri*	
S45710.1	20198	NI	Emily Bay*	*Pterodroma solandri*	MH828429
S45710.2	20199	NI	Emily Bay	*Pterodroma solandri*	
S45710.3	20200	NI	Emily Bay*	*Pterodroma solandri*	MH828430
S45710.4	20201	NI	Emily Bay	*Pterodroma solandri*	
S45699.2	20202	NI	Emily Bay*	*Pterodroma pycrofti*	MH828431
S45701.1	20203	NI	Emily Bay*	*Pterodroma pycrofti*	MH828432
S45705.2	20204	NI	Emily Bay*	*Pterodroma pycrofti*	MH828433
S45706.2	20205	NI	Emily Bay	*Pterodroma pycrofti*	
S45707.2	20206	NI	Emily Bay*	*Pterodroma pycrofti*	MH828434
S45709.1	20207	NI	Emily Bay*	*Pterodroma pycrofti*	MH828435

* = successful ancient DNA extraction and sequencing.

Bioinformatics

Sequenced reads were demultiplexed using SABRE (https://github.com/najoshi/sabre) via the unique 5' and 3' barcodes and processed using the Paleomix pipeline v1.2.12 (Schubert *et al*., 2014). Within Paleomix, adapter sequences were removed and paired end reads merged using ADAPTER REMOVAL 2.1.7 (https://github.com/MikkelSchubert/adapterremoval). Low quality bases were trimmed (Phred20 – minquality = 4) and merged. Reads shorter than 25 bp were discarded (minlength = 25). Read quality was visualised before and after adapter trimming using fastQC v0.11.5 (https://github.com/chgibb/FastQC0.11.5/blob/master/fastqc). Merged reads were mapped against the mitochondrial Cytochrome *b* gene of *P. solandri* (GenBank accession: KX123188.1) using BWA v0.7.15 (aln -l 1024, seed inactivated; -n 0.01, -o 2; https://github.com/lh3/bwa/releases), with minimum mapping quality set at 25. Consensus sequences were called with Geneious v.10.1.3 (http://www.genious.com), with a consensus threshold set to 85% and minimum depth 4. Sequencing reads from our extraction blank controls were not mappable to the reference.

Statistical analyses

A phylogeny was built using all Cytochrome *b* sequences of modern samples (n=176, (Lombal *et al*., 2017)) and ancient DNA samples successfully sequenced. Homologous data were included from *Pterodroma pycrofti* (GenBank accession: MH828447), *Pterodroma neglecta* (GenBank accession: U74341), *Pterodroma nigripennis* (GenBank accession: U74343), *Pterodroma cervicalis* (GenBank accession: EU979553), *Ardenna pacifica* (GenBank accession: AF076088) and *Ardenna carneipes* (GenBank accession: KY443837) given that they breed or are thought to have previously bred on Norfolk Island (Hermes *et al*., 1986; Holdaway and Anderson, 2001). Homologous data from *Pterodroma brevipes* (GenBank accession: MH828446), *Pterodroma leucoptera* (GenBank accession: MK327609),

Pterodroma defilippiana (GenBank accession: MK327608), *Pterodroma arminjoniana* (GenBank accession: GQ328986), *Pterodroma heraldica* (GenBank accession: GQ328988), *Pterodroma cookii* (GenBank accession: U74345), *Pterodroma longirostris* (GenBank accession: U74344), *Pterodroma hypoleuca* (GenBank accession: AF076079), *Pterodroma axillaris* (GenBank accession: U74342) and *Ardenna bulleri* (GenBank accession: AF076081) were also added to our dataset as a mean to assign sequences to other potential candidate contemporary species. Homologous data from *Onychoprion fuscatus* (=*Sterna fuscata*) (GenBank accession: AY631305) was used as an outgroup.

We used Akaike Information Criterion (AIC) scores generated in jModeltest v2.1.10 (Darriba *et al*., 2012) to identify the best fit candidate model of nucleotide evolution (TVM+I+G). This model was employed in PhyML v3.0 (Guindon and Gascuel, 2003) to estimate the maximum likelihood topology, using a BioNJ starting topology and nearest neighbour interchange tree rearrangement. The robustness of the tree was evaluated using 100 bootstrap replicates. A haplotype network was built using the TCS method as implemented in PopART (Leigh and Bryant, 2015) for the same data. Estimates of pairwise population differentiation (F_{st}, G_{st}) among Lord Howe Island (modern and ancient samples), Phillip Island and Norfolk Island were determined using SPADS v 1.0 (Dellicour and Mardulyn, 2014). The statistical significance of F_{st} and G_{st} values was assessed by recalculation based on 10,000 random permutations of individuals among islands.

Results

A total of 22 subfossil samples were successfully sequenced for Cytochrome *b* (720 bp; Table 5 – 1). The subfossil samples appeared in three clades in the phylogenetic tree. Eleven of the ancient samples, and morphologically identified as *P. solandri* (five from Lord Howe Island

and six from Norfolk Island) formed a clade with 176 modern *P. solandri* samples (C1 in Figure 5 – 2). The remaining subfossil samples in the phylogeny were all collected on Norfolk Island. Ten subfossil bone samples, including five morphologically identified as *P. pycrofti* (20202, 20203, 20204, 20206, 20207) and five morphologically identified as *Pterodroma* but not able to be placed into species (17915, 17917, 17922, 17923, 17926), formed a clade with three other species of *Pterodroma* (C2 in Figure 5 – 2). The remaining subfossil sample, initially morphologically identified as *P. solandri* (20200), clustered with species of Ardenna (C3 in Figure 5 – 2); it is a fragment of a humerus which is morphologically similar to both *P. solandri* and *Ardenna pacifica* but fits *A. pacifica* better, so it was evidently misidentified originally – many post-cranial bones of *Ardenna pacifica* are quite similar to those of the similar-sized *P. solandri* – (A. Tennyson pers. Obs. 2018). We do not consider it further in this study.

A haplotype network was built for 187 *P. solandri* samples (176 modern and 11 ancient samples) including all individuals morphologically identified as *P. solandri* (except the sample affiliated with *Ardenna*; see above). The relationships between the 21 haplotypes are shown in Figure 5 – 3. Four out of the five ancient samples collected on Lord Howe Island (17913, 17914, 17918, 17920) exhibited the most frequent haplotype observed in modern samples (Hap_6), with the remaining Lord Howe ancient sample (17919) representing a unique haplotype one mutation away (Hap_21; Figure 5 – 3). Five out of the six ancient *P. solandri* sequences from Norfolk Island (20192, 20193, 20195, 20196, 20198) also exhibited Hap_6. One sample (20194) represented a unique haplotype (Hap_ 20) that was separated from haplotype 6 by 17 mutations (Figure 5 – 3). Low *F*-statistic ($F_{st} = 0.017, p > 0.05$) and lack of phylogeographic signal ($G_{st} = 0.028, p > 0.05$) indicate no significant genetic differentiation among colonies

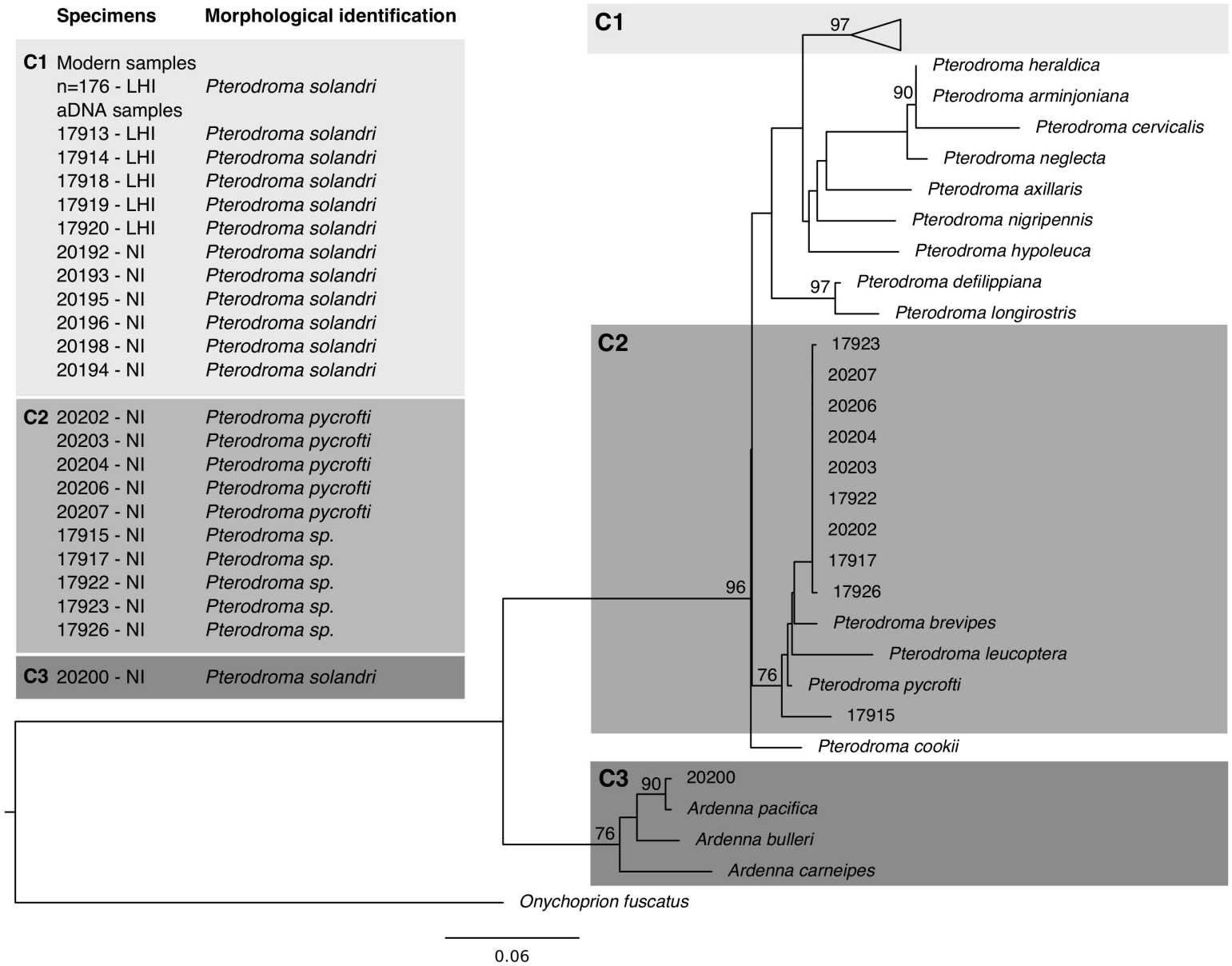
Specimens
Morphological identification
C1 Modern samples
n=176 - LHI
Pterodroma solandri
aDNA samples
17913 - LHI
Pterodroma solandri
17914 - LHI
Pterodroma solandri
17918 - LHI
Pterodroma solandri
17919 - LHI
Pterodroma solandri
17920 - LHI
Pterodroma solandri
20192 - NI
Pterodroma solandri
20193 - NI
Pterodroma solandri
20195 - NI
Pterodroma solandri
20196 - NI
Pterodroma solandri
20198 - NI
Pterodroma solandri
20194 - NI
Pterodroma solandri
C2 20202 - NI
Pterodroma pycrofti
20203 - NI
Pterodroma pycrofti
20204 - NI
Pterodroma pycrofti
20206 - NI
Pterodroma pycrofti
20207 - NI
Pterodroma pycrofti
17915 - NI
Pterodroma sp.
17917 - NI
Pterodroma sp.
17922 - NI
Pterodroma sp.
17923 - NI
Pterodroma sp.
17926 - NI
Pterodroma sp.
C3 20200 - NI
Pterodroma solandri
C1
97
Pterodroma heraldica
90
Pterodroma arminjoniana
Pterodroma cervicalis
Pterodroma neglecta
Pterodroma axillaris
Pterodroma nigripennis
Pterodroma hypoleuca
97
Pterodroma defilippiana
Pterodroma longirostris
C2
17923
20207
20206
20204
20203
17922
20202
17917
96
17926
Pterodroma brevipes
Pterodroma leucoptera
76
Pterodroma pycrofti
17915
Pterodroma cookii
C3
90
20200
Ardenna pacifica
76
Ardenna bulleri
Ardenna carneipes
Onychoprion fuscatus
0.06

Figure 5 – 2 Maximum likelihood tree of the Cytochrome *b* gene in *Pterodroma solandri*, including modern and (n = 176) ancient samples (n = 22). The tree is drawn to scale, with branch lengths measured as the number of substitutions per site. All positions containing gaps and missing data were eliminated. Only bootstrap values >70% are indicated on the cladogram.

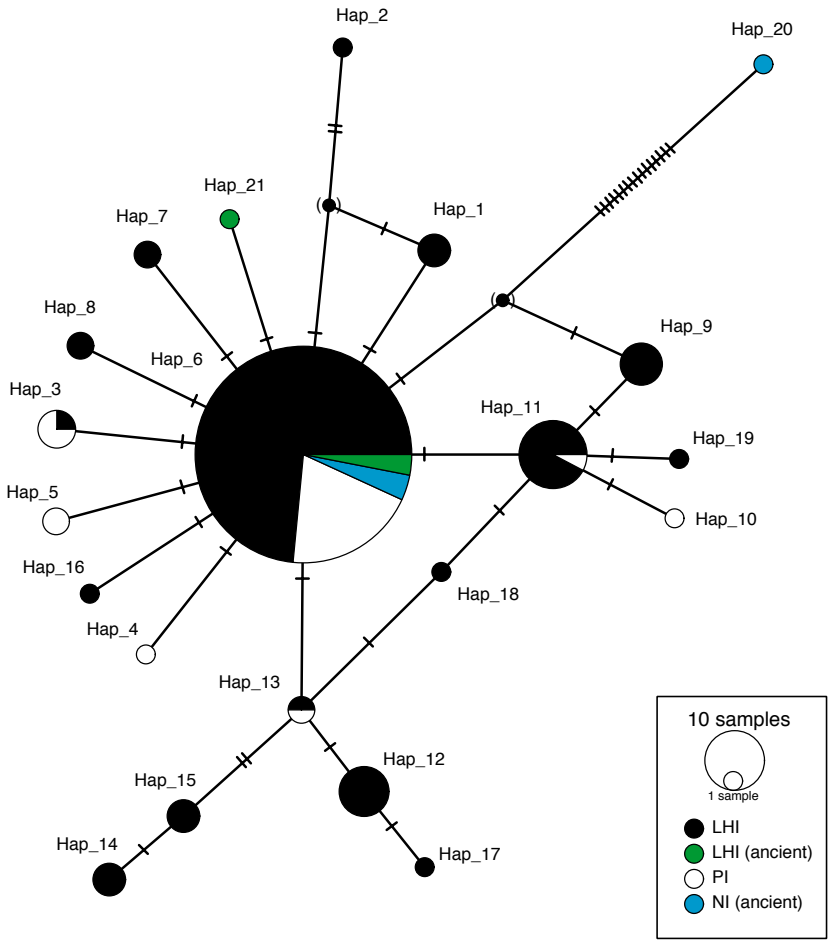

Figure 5 – 3 Haplotype network of *Pterodroma solandri* Cytochrome *b* based on the TCS algorithm. Haplotypes are represented by circles, where the size of each circle is proportional to the frequency of the corresponding haplotype. Lines on connecting branches represent mutations. Black: modern Lord Howe Island individuals. Green: ancient Lord Howe Island individuals. White: modern Phillip Island individuals. Blue: ancient Norfolk Island individuals. () = inferred haplotype.

One additional haplotype network was built for the second clade C2 including 10 ancient samples (20202, 20203, 20204, 20206, 20207, 17915, 17917, 17922, 17923, 17926) and three *Pterodroma* species (*P. pycrofti*, *P. brevipes*, *P. leucoptera*) (C2 in Figure 5 – 2). With the exception of 17915, all ancient samples exhibited the same haplotype that was separated from *P. pycrofti* and *P. brevipes* by one different mutation (Hap_3; Figure 5 – 4). The specimen 17915 showed a distinct haplotype separated from the other specimens by one mutation (Hap_5; Figure 5 – 4).

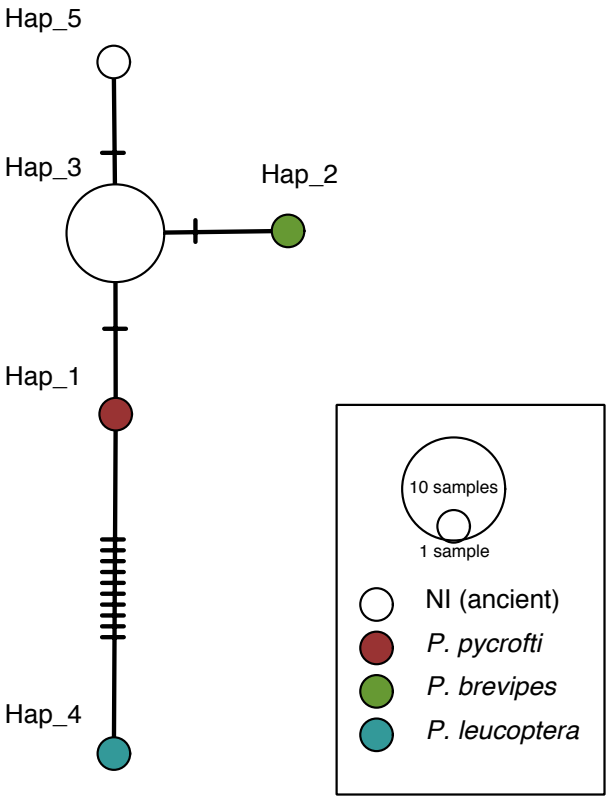

Figure 5 – 4 TCS haplotype network of *Pterodroma* from Norfolk Island (Clade 2) (Cytochrome *b*). Haplotypes are represented by circles, where the size of each circle is proportional to the frequency of the corresponding haplotype. Lines on connecting branches represent mutations. White: subfossil bones from Norfolk Island morphologically identified as *Pterodroma pycrofti*.

Discussion

DNA sequences from heterochronous samples consisting of paleontological bones from Norfolk Island and Lord Howe Island, and those representing the entire current breeding range of *P. solandri,* are dominated by the same mitochondrial DNA variant. The large number of rare haplotypes at Lord Howe also indicates that this population has persisted for a long time. Therefore, the presence of a shared common haplotype indicates a significant level of genetic connectivity between the Norfolk and Lord Howe populations prior to the extinction of the Norfolk population. This new knowledge is valuable for assessing extinction risks of contemporary populations based on their genetic connectivity.

Although the potential for dispersal will likely help the persistence of populations in the short-term, long-term viability is dependent on the intensity of negative processes affecting the population and on the life-history traits of a species. In the case of the providence petrel, the anthropogenic impacts on the Norfolk Island population were strong. The population was estimated at ~1,000,000 breeding pairs before March 1790 when the HMS *Sirius* was wrecked on a reef at Norfolk Island. During the next four months, the shipwreck survivors avoided starvation by slaughtering hundreds of thousands of providence petrels. An estimated 1,600 birds were harvested per night during the four month breeding season, during years 1790 – 1793 (Medway, 2002a). In 1971, an officer of the ship added: 'three or four thousands of birds have been sometimes killed in one night. The convicts senseless and improvident, not only destroyed the birds, its young and its egg, but also the hole in which it burrowed' (Medway, 2002a). This strong selection applied to a procellariiform species, which typically exhibit low fecundity and high longevity, made the Norfolk Island population of providence petrels vulnerable to extinction as threats to the adult population were persistent rather than episodic. Moreover, greater numbers of randomly chosen migrants may be necessary to achieve the

same genetic effect as fewer migrants chosen to maximize contribution to effective population size (Kleiman, 1989; Mills and Allendorf, 1996). Additionally, the origin of migrants, how many individuals migrate, and whether the migrants settle in unoccupied or occupied patches may also affect the benefits of immigration (McCauley, 1991; Lowe and Allendorf, 2010). Hence, the level of gene flow that maintained genetic homogeneity may have been insufficient to be demographically influential (Lowe and Allendorf, 2010), yet also sufficient to inhibit natural selection (Allendorf, 1983), and the population probably went extinct under the influence of subsequent predation and disturbance from invasive mammals such as pigs, which has been documented for other burrowing seabird populations (Hilton and Cuthbert, 2010).

The ancient Norfolk Island petrel that did not exhibit the common contemporary haplotype (20194; Figure 5 –2) possessed 17 private mutations, and may represent another species historically breeding on Norfolk Island or a vagrant. Only four *Pterodroma* species are reported to currently breed on Norfolk – *P. solandri, P. cervicalis, P. neglecta* and *P. nigripennis* (Hermes *et al*., 1986) – and reference sequences from all are represented in my analysis. However, the list of breeding species might be incomplete, and it is possible that another unrecognized species bred on Norfolk Island before European settlement (see appendix in Holdaway and Anderson, 2001). Taxonomically cryptic species have been widely observed in insular regions (Murphy *et al*., 2011; Saitoh *et al*., 2015). For example, the differences in size and morphology of *Pterodroma* subfossils from the Canary Islands compared with the two current Macaronesian breeding species of *Pterodroma* (Fea's petrel *Pterodroma feae* in the Cape Verde Islands and Madeiran petrel *Pterodroma madeira* in Madeira) indicate the possible existence of a distinct *Pterodroma* in the Canary Islands historically (Rando, 2002). Similarly, morphological and genetic evidence revealed an extinct

Pterodroma species, *Pterodroma imberi*, from the Chatham Islands that was intermediate in size between two extant species (Cooper and Tennyson, 2008; Tennyson *et al*., 2015).

The Norfolk subfossils morphologically described as *P. pycrofti* analyzed in the present study all showed the same haplotype that differed from *P. pycrofti* and *P. brevipes* by one mutation. *Pterodroma pycrofti* and *P. brevipes* bones overlap in size (A. Tennyson; pers. obs.). Our genetic results corroborate morphological identification suggesting that these subfossils may be *P. pycrofti,* or *P. brevipes* or another closely-related, possibly undescribed taxon. However, the dimension and colour pattern of a small *Pterodroma* painted at the time of first European settlement (Hindwood, 1965) fit those of *P. pycrofti* better than those of *P. brevipes*. Moreover, the current breeding distribution of *P. pycrofti* in temperate waters (small islands off the northeastern coast of the North Island of New Zealand (Heather and Robertson, 1996)) fits better with a breeding population on Norfolk than does that of *P. brevipes*, which is restricted to tropical Pacific breeding populations (see (Tennyson and Miskelly, 2012)). Further research should be conducted to address this uncertainty.

Connectivity between providence petrel population suggests that the nocturnal roost arrival observed on Norfolk Island and Phillip Island reflects phenotypic plasticity. Numerous studies have illustrated the importance of behavioural plasticity as a fundamental trait of life history strategies in seabirds living in highly dynamic and variable environment (e.g. Falk *et al*., 2002; Paiva *et al*., 2009; Reed *et al*., 2009). However, it has been suggested that the main factor responsible for nocturnal colony arrival in small Procellariiformes is to avoid predators (Watanuki, 1986; Warham, 1990; McNeil *et al*., 1993; Keitt *et al*., 2004), and while at least one avian predator (*A. fasciatus*) was present at the time of European settlement on Norfolk Island (Medway, 2002a), they are currently absent (Hermes *et al*., 1986). Alternatively,

nocturnal arrival could reflect foraging strategy, as has been observed in other seabird taxa (Baduini, 2002; Dias *et al*., 2012). For example, Cory's shearwaters *Calonectris diomedea* show intraspecific variation in colony arrival depending on the marine region and abundance of prey, and are high flexibility in their daily routines (Dias *et al*., 2012). The likelihood of phenotypic plasticity supports the potential use of Lord Howe individuals to re-establish a providence petrel population on Norfolk Island. This will reduce the extinction risk of the species, restore the input of marine-derived nutrients into the ecosystem (particularly phosphorous), and reverse the susceptibility of Norfolk Island pines (*Araucaria heterophylla*) to the root-rotting fungus *Phellinus noxius* (see Holdaway and Christian, 2010).

My study provides an insight into how rapidly even very large populations can be extirpated despite genetic connectivity among populations, which has significant conservation implications for further studies attempting to predict the resilience of populations based on genetic data. Furthermore, this approach, based on a presently under-exploited potential of ancient DNA studies, can be applied to other taxa to estimate genetic connectivity prior to extinction and identify instances of taxonomically cryptic extinctions. Indeed, humans have had a great impact on global biodiversity, especially on the islands of the Pacific Ocean (Steadman, 1989, 2006; Pimm *et al*., 1994), and Norfolk Island fits the pattern of several other Pacific islands, where early contact by Polynesian settlers resulted in the extinction of the resident bird species. Hence, many replicate examples could be studied in the Pacific to confirm the generality of our findings. Similarly, aDNA analysis can provide a more comprehensive documentation of the extinctions of birds from these localities.

The use of aDNA may also allow for the historical (pre-extirpation or post-extinction) distributions of taxa to be investigated and described. Material already collected and available

in museums can be incorporated into future studies to document the historical faunas of island where extirpations or extinctions are known providing further insights into the relationships between and among islands' populations and their avian communities before human arrival and settlement.

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Chapter 6:

Historical and physical factors dominate biotic processes as determinants of seabird population genetic differentiation

Historical and physical factors dominate biotic processes as determinants of seabird population genetic differentiation

Anicee J. Lombal[1,4], James E. O'Dwyer[1,5], Vicki Friesen[2,6], Eric J. Woehler[2,7] and Christopher P. Burridge[1,8].

[1]School of Natural Sciences, University of Tasmania, Hobart, TAS 7001, Australia. Phone: +61 3 6226 7653.

[2]Department of Biology, Queen's University, Kingston, OL K7L 3N6, Canada. Phone:+ 61 3 533 6617

[3]Institute for Marine and Antarctic Studies, University of Tasmania, Hobart, TAS 7001, Australia. Phone: +61 3 6226 6379

[4]Anicee.Lombal@utas.edu.au

[5]18088076@students.latrobe.edu.au

[6]Vlf@queensu.ca

[7]Eric.Woehler@utas.edu.au

[8]Chris.Burridge@utas.edu.au

Abstract

Understanding factors leading to genetic differentiation of populations is highly desirable to help identify conservation priorities and maintain viability of species. Seabirds provide a tractable group within which to identify these factors, given their widespread distribution in marine habitats and the abundance of ecological and genetic studies already conducted on this group. In this study, I evaluated a candidate set of generalized linear models (GLMs) to identify determinants of population differentiation in mitochondrial DNA (mtDNA) for 73 seabird species. Lack of mutation-drift equilibrium observed in 19% of species coincided with lower estimates of genetic differentiation, suggesting that dynamic demographic histories are highly influential. Presence of land across the sampling range of species, or sampling of breeding colonies representing ice-free Pleistocene refuge zones, were the best predictors of genetic differentiation within Tropical and Southern Temperate species, respectively, and was supported phenotypic variation. Conversely, biotic factors such as variation in non-breeding distributions among colonies, population size and International Union for Conservation of Nature threat status were not significant predictors of population genetic differentiation. In light of these results, I recommend that mtDNA studies should consider potentially influential historical factors to avoid overestimating the impact of biotic determinants when identifying conservation genetic priorities of seabird species.

Keywords: population genetics, gene flow, historical fragmentation, mutation-drift equilibrium, IUCN.

Introduction

Predicting the extent of genetic differentiation among populations based on factors likely to influence gene flow, such as presence of pelagic larvae versus direct development in fishes (Kyle and Boulding, 2000; Dawson *et al*., 2014), or spatial and temporal dynamics of seed movement (Sork *et al*., 1999), is highly desirable for identifying conservation priorities and maintaining viability of species (DeSalle and Amato, 2004). Genetically differentiated populations are often of elevated conservation concern because they may experience reduction of genetic variability through genetic drift and inbreeding (Frankham, 1996), which may decrease their adaptability to future environmental variation (Frankham *et al*., 2002). Knowledge derived from genetic analyses of natural populations has thus been used to advance our understanding of factors influencing genetic differentiation (Wright, 1931; Weir and Cockerham, 1984). However, it is now accepted that the assumptions of models for quantifying population genetic structure may provide unrealistic representations of contemporary gene flow (Whitlock and McCauley, 1999; Pearse and Crandall, 2004). This is particularly true for species at high latitudes because most studied populations are likely to have experienced bottlenecks, range fragmentation or other perturbances during Pleistocene climatic transitions (Hewitt, 1996, 2004), which can bias the quantification of population genetic structure (e.g., F_{st}, Φ_{st}) (Wright, 1931).

Seabirds represent an ideal model system in which to investigate the determinants of population genetic structuring. Firstly, although relatively few in number, seabirds occur in all seas and oceans worldwide (Croxall *et al*., 2012), and therefore have been exposed to a variety of environmental conditions and histories that may have influenced genetic differentiation. Moreover, the potential of seabirds as indicators of marine conditions is now widely recognized (Montevecchi, 1993; Piatt *et al*., 2007; Parsons *et al*., 2008; Montevecchi *et al*., 2012), but

knowledge of seabird movements, and hence genetic differentiation, is also relevant to our understanding of their responses to changes in the marine environment. Seabirds are also more threatened than other group of birds, and their status has deteriorated rapidly over recent decades (Croxall *et al*., 2012). Consequently, seabirds are highly studied, such that background information on candidate determinants of genetic structuring is often available (e.g. estimates of population size, telemetry studies of migratory movements). Furthermore, seabirds have been genetically well-studied because the discrete breeding distributions of many seabirds (colonies) expedites robust sampling for population genetic analysis (Friesen *et al*., 2007), yet there is still substantial benefit in the successful prediction of genetic structuring without the need for fieldwork and genotyping. Finally, factors influencing population genetic structuring in seabirds have already been hypothesized (Friesen *et al*., 2007; Friesen, 2015), providing a framework from which to expand our knowledgebase.

Genetic structuring in seabirds may be susceptible to climatic oscillations during the Pleistocene, particularly for high latitude taxa, with suggestions of genetic homogenization during species persistence in a single climatic refugium (e.g. Moum and Arnason, 2001; Wojczulanis-Jakubas *et al*., 2015) and subsequent widescale expansion (Jakobsson *et al*., 2014). However, enhanced population genetic differentiation is also possible if sequential bottlenecking accompanied post-glacial population expansion (Hewitt, 2000). Given that areas today glaciated during the Pleistocene may have only been accessible during the last 10,000 years (Jakobsson *et al*., 2014), the effects of migration, mutation and drift may not yet be at equilibrium, particularly given the relatively large population size of many seabirds (Fitzpatrick *et al*., 2012; Wojczulanis-Jakubas *et al*., 2015). Therefore, measures of population genetic differentiation may not entirely reflect contemporary processes given legacies of historical climates.

Although many species to travel long distances over water (Burger and Shaffer, 2008), seabird colonies separated by land show high levels of genetic differentiation (Friesen, 2015). For example, the Isthmus of Panama, with a minimum width of only ~30 km, prevents dispersal among colonies of sulids – gannets and boobies (Morris-Pocock *et al*., 2010; Steeves *et al*., 2003). Similarly, other physical but non-terrestrial barriers have been inferred to restrict gene flow and lead to genetic divergence among seabird colonies (Friesen, 2015), such as oceanographic fronts in the temperate Southern Hemisphere (Younger *et al*., 2016; Munro and Burg, 2017). The patchy distribution of suitable nesting habitats for seabirds (e.g. availability of islands in the oceans) often results in colonies separated by large marine distances (Coulson, 2001), which potentially restricts gene flow among colonies. However, Friesen (2007) showed that geographic distance provided a weak explanation of population genetic differentiation in seabirds, and several species show strong genetic differentiation within single islands or archipelagos (Burg and Croxall, 2001; Smith and Friesen, 2007; Wiley *et al*., 2012). However, genetic structure can also exist in the apparent absence of physical barriers to gene flow, suggesting that biotic factors are also important (Burg and Croxall, 2001; Friesen, 2015; Friesen *et al*., 2007; Yeung *et al*., 2009).

Although several biotic factors have been suggested to affect genetic divergence among seabird colonies, they provide only a weak explanation of the extent of population genetic differentiation (Friesen *et al*., 2007; Friesen, 2015). Philopatry has been proposed as a major predictor of genetic structure (Friesen, 2015; Warham, 1990). However, Coulson (2016) showed that many banding studies may have overestimated natal philopatry, which makes meaningful tests of relationship between philopatry and genetic structure difficult. While Friesen (2015) proposed that differences in non-breeding distributions can predict genetic differentiation (e.g. Burg *et al*., 2003; Clucas *et al*., 2014; Gangloff *et al*., 2013), other studies

found no such relationship (e.g. Lombal *et al*., 2018, see Chapter 3; Quillfeldt *et al*., 2017). Intra-specific competition for food may result in greater foraging distances in larger seabird colonies (Ashmole, 1971; Lewis *et al*., 2001), potentially promoting gene flow. Larger colonies will also experience less genetic drift. Indeed, population genetic structure is less common in species with more than 10^6 breeding pairs (Friesen *et al*., 2007).

While the general impact of each of these ecological factors on genetic structure among seabird colonies has been assessed (Friesen *et al*., 2007), little is known about the interactions among these factors and historical processes as determinants of current seabird population genetic differentiation.

Morphological and phenological differences among populations inhabiting different environments may predict genetic isolation among colonies (Avise, 2000). However, in seabirds, these factors are not always accompanied by genetic structure or reciprocal monophyly of mitochondrial lineages (Liebers and Helbig, 2002; Lombal *et al*., 2017; Wiley *et al*., 2011), especially at high latitudes (Liebers and Helbig, 2002; Moum and Arnason, 2001). For example, in the lesser black-backed gull *Larus fuscus*, the divergence between taxonomic *L. f. heuglini* and *L. f. fuscus* is reflected in behavioral and ecological segregation, but reciprocal monophyly is lacking for mtDNA (Liebers and Helbig, 2002). This suggests that, in seabirds, recently separated populations may lack population genetic differentiation due to retained ancestral variation (Friesen *et al*., 2007). Any apparent relationship between phenotypic and genetic divergence also needs to consider the potential confounding factors.

Given that small, isolated populations are often of elevated conservation priority, species of higher threat status (e.g., International Union for Conservation of Nature, IUCN) might be expected to exhibit greater population genetic structuring. A negative relationship exists between threat status and absolute genetic diversity within populations (Spielman, Brook & Frankham, 2004), and other potential relationships with threat status such as body mass and human encroachment have also been investigated (Jetz & Freckleton, 2015). However, despite the wealth of genetic studies conducted on threatened species in an effort to identify logical spatial units for conservation, the extent to which IUCN status captures population genetic differentiation—and hence elevated conservation priority of populations—is unknown.

While previous reviews have qualitatively assessed several predictors of genetic differentiation among seabird colonies (Friesen *et al*., 2007; Friesen, 2015), here I conducted multiple generalized linear models (GLMs) based on mtDNA variation to investigate this topic. To identify historical contributors to contemporary population genetic differentiation, I investigated the impact of demographic change and sampling from putative climatic refugia. I also investigated the contribution of physical barriers to population genetic differentiation with respect to land barriers, oceanographic fronts, and geographic distance. To identify biotic contributors to population genetic differentiation, I tested for relationships with population size, IUCN status, and variation in non-breeding distributions, breeding phenology and morphology. I also tested whether any inferred relationships could be confounded by other factors.

Materials and Methods

Literature search and data collection

Peer-reviewed studies reporting population genetic data for seabird species were obtained from a search of Web of Science using the terms: 'seabird' and 'F_{st}'or 'Φ_{st}' or 'population genetic structure'. Where multiple studies existed for the same species, one study per species was selected based on three hierarchical criteria: 1) availability of global *F*-statistic (F_{st} or Φ_{st}), 2) availability of mitochondrial DNA (mtDNA) sequences on GenBank and 3) largest sampling range. Data were solicited from the authors of papers for which mtDNA sequences, haplotype frequencies or global *F*-statistics were not available in the literature. When available on GenBank, mitochondrial DNA sequences (Cytochrome *b*, Control Region (CR), COX1, NADH or ATPase) were collected and combined in one fasta file per species following haplotype frequencies for each sampled colony within species.

Although mtDNA has been extensively used to quantify population genetic differentiation of animals (Avise, 2004), using mtDNA to test potential predictors of differentiation has several challenges. 1) Different mtDNA genes have different mutation rates (Ballard and Whitlock, 2004), which complicates comparisons among studies. One remedy is to include 'gene' as a predictor of genetic structure in statistical analyses. 2) Metrics of population genetic differentiation (e.g. F_{st}) are influenced by genetic variation within populations (Hedrick, 2005). Measures of variation at the DNA level (h, π) could be included as a predictor of genetic structure in statistical analyses. 3) Differences among metrics of population genetic variation *per se* may lead to bias in multi-species comparison (Whitlock, 2011). Metrics based on haplotype frequency alone, such as F_{st} may reflect different histories from those incorporating mutational distance among haplotypes such as Φ_{st} (Weir and Cockerham, 1984;

Slatkin and Barton, 1989). Hence, it is essential to compare each class of index independently.

For each study, the following data were collected: genetic marker, sample size, number and geolocation of sample sites, population size in breeding pairs, IUCN Status (http://www.iucnredlist.org/), and maximum marine distance between sampled colonies as inferred through the National Hurricane Centre's distance calculator (http://www.nhc.noaa.gov/gccalc.shtml). Sample sizes implemented in the generalized linear models GLMs were adjusted to the number of sequences available on GenBank as used in the calculation of *F*-statistics where it differed from the number of sequences reported in the publication. *F*-statistics (F_{st} and Φ_{st}), haplotype diversity (*h*), nucleotide diversity (π), and Tajima's D test were calculated using DNAsp 5.10.01 (Librado and Rozas, 2009) and SPADS v1.0 (Dellicour and Mardulyn, 2014). Migratory status, distinction of non-breeding distribution in migratory species, and distinction of non-breeding distribution regardless of migratory status were tested, as were differences in morphology and breeding phenology as recorded from the literature (all were binomial factors). Non-breeding distributions for migratory species were obtained from telemetry studies in some instances.

Because the species studied share parts of their phylogenetic history, they may not represent independent observations of any relationship between predictors and the response variable. For example, in flying birds, the load-carrying capacity decreases with increasing body mass (Hedenström, 1992), which means that the maximum flight range decreases with increasing size (Alerstam *et al*., 2003), although large birds compensate by possessing relatively long wings (Rayner, 1988). Hence, the correlation between body size – a phylogenetically conserved trait – and migration (Hedenström, 1992; Hedenström and Alerstam, 1998) may

lead to false inferences regarding body size and or migration and genetic differentiation if only one factor is influential. To address this possibility, we tested whether taxonomic family predicted F_{st}.

For Southern Temperate species, presence of sample colonies in refuge zones was recorded, where refuge zones were defined a priori following previous seabird studies: islands located north of the Subtropical oceanographic Front (Gough Island, Tristan da Cunha and Amsterdam Island), the Falklands Islands, New Zealand Sub Antarctic Islands (Auckland and Antipode Islands) (de Dinechin *et al*., 2009; Techow *et al*., 2009). For Southern Temperate species, one additional predictor of genetic structure was tested: the sampling range crossing the Antarctic Polar Front (APF) or the Subtropical Front (SF) (see Figure 6 – 1 in the section 'Results') (Younger *et al*., 2016; Munro and Burg, 2017). To test these predictors of genetic differentiation among colonies according to broad geographical regions, species were classified into four categories. Species with > 50% of their sampled range between the Tropic of Cancer 23° 27'N and the Tropic of Capricorn 23° 27'S were defined as Tropical species; between latitudes 23° 27' N and 66° 30'N were defined as Northern Temperate species; between latitudes 23° 27'S and 66° 30'S were defined as Southern Temperate species and species with approximately equal sampling between 23° 27'N – 66° 30'N and 23° 27'S – 66° 30'S were defined as Northern and Southern species.

Statistical analysis

I first tested mutation-drift equilibrium (Tajima's D) as a predictor of F_{st} (historical factor; model M1). As genetic structure among colonies can create spurious demographic signals (population expansion — significant negative Tajima's D), *post hoc* neutrality tests were performed for species exhibiting both a significant F_{st} and a significant Tajima's D, as

suggested by Chikhi *et al*. (2010). For these species, Tajima's D and Fu and Li's D tests (Fu and Li, 1993) were conducted for each sampled colony and each genetically distinct group of colonies as defined in the literature. If a demographic signature is still detected, it can be concluded that it is not erroneously a product of genetic differentiation among colonies. All subsequent analyses are based on species exhibiting mutation-drift equilibrium.

I then tested a model M1' of predictors and potentially confounding variables, including genetic marker, taxonomic family, number of sample sites, sample size, population size, IUCN status, geographic region, marine distance between the most distant sampled colonies, haplotype diversity (*h*) and nucleotide diversity (π). Differences between populations in breeding phenology, morphology, migratory status and non-breeding distributions were also tested as predictors of F_{st}. Given that these predictors showed fewer degrees of freedom than the others (see section 'Results'), they were tested as separate models (M2, M3 and M4, respectively) where missing values were discarded (function *na.omit* in glm). Three individual tests were conducted in model M4 (M4a–c). In M4a the predictor was composed of three categories: 1) migratory species (M), 2) partially-migratory species (only certain colonies or individuals migrate; M^{p}), 3) year-resident species (R). In M4b, the predictor was composed of two categories: 1) migratory species exhibiting different non-breeding distributions among colonies (M^{Y}; where Y = differences in non-breeding distributions) combined with year-resident species (R), 2) migratory species exhibiting an overlap of non-breeding distributions (M^{N}; where N = no differences in non-breeding distributions). As the presence or absence of difference in non-breeding distributions may not influence genetic differentiation independent of migratory status (M vs. R), in M4c the predictor was composed of two categories: 1) M^{Y}, 2) M^{N}.

Six homologous GLMs each were built for Northern Temperate species (N1 – N4a–c), Tropical species (T1 – T4a–c) and Southern Temperate species (S1 – S4a–c). For Northern Temperate and Tropical species, I tested the presence of land separating sample colonies as an historical predictor of genetic structuring. For Southern Temperate species, I tested the presence of sample colonies on islands identified as refuge zones or the sampling range of species crossing the APF or the SF as predictors of F_{st}. Moreover, I explored potential hierarchy of genetic partitioning among Northern Temperate populations according to the location of separation (e.g. Mediterranean vs. Atlantic or Pacific vs. Atlantic) and among Southern Temperate populations according to their presence in different refuge zones (e.g. the Falkland Islands or Gough Island), which may reflect different susceptibility to historical events. Species sampled in the Northern and the Southern Hemisphere (n = 2) were discarded from the dataset and described separately.

Statistical analyses were performed using the R v1.1.383 package of programs. There was no significant difference between F_{st} and Φ_{st} where both were reported (paired ANOVA: $F = 0.003$, d.f. = 1, $p = 0.956$). Hence, I conducted the subsequent analyses with F_{st} only. I fit all the GLM models with F_{st} as a response variable to different predictors with a) a logit link function and a binomial error distribution, where F_{st} with $p < 0.05$ was coded as 0 and F_{st} with $p > 0.05$ was assigned a value of 1 and b) F_{st} implemented as a continuous variable, with $F_{st} <$ 0 changed to 0, and 0.01 added to all values to permit log transformation. To improve approximation of normality of F_{st}, I used the log transformation of Rousset (1997) such that the final variable was $\log((F_{st} + 0.01)/(1-(F_{st} + 0.01))$.

Changes in model deviance (χ^2) and the Akaike information criterion (AIC; Akaike, 1974) were used to assess the significance of predictors and to define the best fit combination of

predictors for all GLMs. The AIC highest ranked models are those explaining the most variance in the data yet excluding unnecessary predictors that cannot be justified for inference on the basis of the data (Burnham and Anderson, 2001). I explored how defined predictors fit the data including interactions between them. Only the significant predictors are reported. Only the results of models improving AIC are reported in the successive categories of predictors tested in each model (single predictor – single additional predictor – multiple additional predictors – interactions). ANOVAs, simple and multiple linear regressions and χ^2 tests were conducted on significant predictors of F_{st} to examine the strength of relationships between predictors and genetic structure.

Visualization of barriers to dispersal

To visualize the geographic location of significant genetic differentiation within seabird species I used the Monmonier's (1973) maximum distance algorithm (Manni *et al*., 2004), which identifies the areas where a given variable shows an abrupt rate of change as implemented in BARRIER v2.2 (Manni & Guérard, 2004). This method is based on the pairwise F_{st} matrix among colonies. I limited the number of genetic barriers to the four highest F_{st} of the pairwise matrix obtained for each species ("a", "b", "c" and "d") to allow a better visualization of our data. The genetic barriers obtained were manually added to a 2D kernel density contour of latitude (Y axis) and longitude (X axis) of cumulated sample sites built with the MASS R package to obtain a visual approximation of the locations of barriers to gene flow among seabird colonies relative to the total number of sample sites.

Results

Literature searches and data transformations

My literature research resulted in 151 genetic studies representing 91 seabird species. After selection based on three hierarchical criteria described above and following positive replies from four authors, I achieved at least one global *F*-statistic (F_{st}, Φ_{st}) from 73 species representing six seabird families: Procellariidae, Sulidae, Phalacrocoracidae, Spheniscidae, Laridae, Alcidae (Table 6 – 1). The species were defined without regard to any subsequent elevation of distinct genetic clusters to officially recognized species, as the aim of this study was to identify predictors of genetic structuring in species as recognized prior to genetic analysis (i.e. predicting genetic structuring among colonies regardless of cryptic errors in taxonomy). The current taxonomy of all 73 species based on Handbook of the Birds of the World (HBW) and Birdlife Taxonomic Checklist 2017 is reported individually in Supplementary Information SI. 6 – 1. Data from 538 sample sites were collected (Supplementary Information SI. 6 – 1). The two most distant sample sites and the distance between these sites (km) per species are reported in Supplementary Information SI. 6 – 1 and Table 6 – 1 (Log(dist)), respectively.

Table 6 – 1 Factors potentially contributing to genetic differentiation within seabird species and indices of genetic structure (F_{st}, Φ_{st}), genetic diversity (h, π) and Tajima's D.

Family	n°	Species	Common name	IUCN[a]	Pop size[b]	Sites	n	Region[c]	Log(dist)[d]	Marker	bp	F_{st} [e]	Φ_{st} [f]	π	h	D[g]
Procellariidae	**1**	*Ardenna carneipes*	flesh-footed shearwater	VU	4.87	13	148	S	3.60	Cytb	857	0.747*	0.722*	0.004	0.697	-0.02
Procellariidae	**2**	*Ardenna mauretanicus*	balearic shearwater	CR	3.47	10	113	N	2.40	Cytb	886	0.145*	0.214*	0.005	0.806	-0.54
Procellariidae	**3**	*Ardenna tenuirostris*	short-tailed shearwater	LC	7.36	11	335	S	2.90	REs[s]	-	0.284*	-	-	-	-
Procellariidae	**4**	*Calonectris diomedea*	Cory's shearwater[δ]	LC	5.39	27	241	N	3.40	CR	293	0.560*	0.549*	0.037	0.979	0.10
Procellariidae	**5**	*Diomedea exulans*	wandering albatross	VU	3.78	7	79	S	3.74	CR	234	0.639*	0.638*	0.033	0.971	-0.57
Procellariidae	**6**	*Fulmarus glacialis*	northern fulmar[δ]	LC	7.30	17	134	N	3.77	CR	228	0.783*	0.779*	0.041	0.933	0.21
Procellariidae	**7**	*Halobaena caeruela*	blue petrel	LC	6.18	2	27	S	3.81	Cytb	889	0.008	0.040	0.001	0.336	-1.54
Procellariidae	**8**	*Hydrobates pelagicus*	European storm petrel	LC	5.69	5	65	N	3.30	Cytb	910	0.937*	0.928*	0.004	0.700	0.70
Procellariidae	**9**	*Macronectes giganteus*	southern giant petrel	LC	4.48	12	74	S	3.48	Cytb	752	0.572*	0.599*	0.005	0.785	0.48
Procellariidae	**10**	*Macronectes halli*	northern giant petrel	LC	4.04	9	51	S	3.65	Cytb	752	0.207*	0.215*	0.002	0.721	-0.46
Procellariidae	**11**	*Oceanodroma castro*	band-rumped storm petrel[δ]	LC	5.17	7	383	NS	4.18	CR	448	0.741*	0.746*	0.035	0.979	-0.65
Procellariidae	**12**	*Oceanodroma l. leucorhoa*	Leach's storm petrel[Ψ]	VU	7.00	9	103	N	3.60	CR	357	0.283*	0.602*	0.006	0.783	0.14
Procellariidae	**13**	*Pachyptila belcheri*	thin-billed prion[Ψ]	LC	6.54	2	32	S	3.90	Cytb	889	0.326*	0.315*	0.002	0.895	-1.35
Procellariidae	**14**	*Pachyptila desolata*	Antarctic prion	LC	7.39	2	32	S	3.81	Cytb	889	-0.026	-0.009	0.002	0.702	-2.01*
Procellariidae	**15**	*Pachyptila turtur*	fairy prion	LC	6.69	3	61	S	2.30	REs[s]	-	0.100	0.228*	0.095	0.551	-1.50
Procellariidae	**16**	*Pelagodroma marina*	white-faced storm-petrel[δ]	LC	6.30	3	63	NS	3.60	CR	522	0.893*	0.895*	0.050	0.969	1.28
Procellariidae	**17**	*Phoebastria immutabilis*	Laysan albatross	NT	5.77	11	358	N	3.98	CR	189	0.135*	-0.005	0.045	0.989	-0.87
Procellariidae	**18**	*Phoebastria nigripes*	black-footed albatross	NT	4.81	4	139	N	3.54	CR	609	0.854*	0.914*	0.003	0.548	0.97
Procellariidae	**19**	*Procellaria aequinoctialis*	white chinned petrel	VU	6.08	5	90	S	3.60	Cytb	599	0.602*	0.761*	0.005	0.781	-0.40
Procellariidae	**20**	*Pterodroma arminjoniana*	trindade petrel	VU	3.04	2	47	N	3.96	Cytb	995	0.265*	0.237*	0.004	0.703	-0.45
Procellariidae	**21**	*Pterodroma cookii*	Cook's petrel	VU	5.47	2	45	S	3.00	COX1	677	0.758*	0.728*	0.001	0.612	0.01
Procellariidae	**22**	*Pterodroma mollis*	gadfly petrel[δ]	NT	3.00	3	210	N	3.00	Cytb	872	0.948*	0.953*	0.012	0.779	1.09
Procellariidae	**23**	*Pterodroma phaeopygia*	Galápagos petrel	CR	4.00	5	32	T	2.00	Cytb	1143	0.044	0.040	0.001	0.857	-1.79*
Procellariidae	**24**	*Pterodroma sandwichensis*	Hawaiian petrel	VU	4.00	2	80	N	2.30	Cytb	495	0.450*	0.447*	0.005	0.812	-0.27
Procellariidae	**25**	*Pterodroma solandri*	providence petrel	VU	4.47	5	151	T	3.00	Cytb	872	0.022	0.018	0.001	0.534	-2.19**

Procellariidae	**26**	*Thalassarche cauta*	shy albatross	NT	4.17	3	30	S	2.30	CR	299	0.082	0.065	0.008	0.860	-1.32
Procellariidae	**27**	*Thalassarche chrysostoma*	grey-headed albatross	EN	5.39	5	50	S	3.86	CR	220	0.032	0.014	0.030	0.994	-1.32
Procellariidae	**28**	*Thalassarche melanophris*	black-browed albatross$^{\delta}$	NT	5.84	6	73	S	3.86	CR	211	0.568*	0.629*	0.039	0.981	-0.74
Procellariidae	**29**	*Thalassarche steadi*	white capped albatross	NT	5.00	3	29	S	1.78	CR	299	0.014	0.025	0.014	0.968	-1.60
Sulidae	**30**	*Sula dactylatra*	masked booby	LC	4.00	4	64	T	3.70	Cytb	450	0.641*	0.614*	0.006	0.593	1.38
Sulidae	**31**	*Sula grandi*	nazca booby	LC	4.47	5	50	T	2.30	Cytb/ND2/COI	780	-	0.127	0.001	0.886	-
Sulidae	**32**	*Sula leucogaster*	brown booby	LC	5.30	11	242	T	3.95	CR	489	0.768*	0.783*	0.062	1.000	0.88
Sulidae	**33**	*Sula nebouxii*	blue-footed booby	LC	5.00	9	154	T	3.48	CR	538	0.047	0.044	0.016	0.993	-1.41
Sulidae	**34**	*Sula sula*	red-footed booby	LC	6.00	10	271	T	3.95	CR	473	0.736*	0.803*	0.049	1.000	0.06
Sulidae	**35**	*Sula variegata*	Peruvian booby	LC	6.25	5	153	T	3.48	CR	540	0.009	-0.002	0.018	0.991	-1.27
Phalacrocoracidae	**36**	*Phalacrocorax aristotelis*	European cormorant	LC	5.00	11	66	N	3.48	NADH	320	0.937*!	0.704*	0.002	0.440	1.14
Phalacrocoracidae	**37**	*Phalacrocorax atriceps*	imperial shag$^{\Psi}$	LC	4.69	11	90	S	3.08	ATPase	657	0.498*	0.388*	0.002	0.756	0.72
Phalacrocoracidae	**38**	*Phalacrocorax auritus*	double-crested cormorant	LC	5.54	23	248	N	3.78	CR	700	0.636*	0.431*	0.035	0.976	0.71
Phalacrocoracidae	**39**	*Phalacrocorax carbo*	great cormorant	LC	6.00	20	231	N	3.48	CR	434	0.131*	0.139*	-	-	-
Phalacrocoracidae	**40**	*Phalacrocorax magellanicus*	rock shag	LC	6.00	12	83	S	3.08	ATPase	657	0.713*	0.815*	0.003	0.542	0.53
Sphenicidae	**41**	*Aptenodytes forsteri*	emperor penguin	NT	5.77	8	226	S	3.48	CR	368	0.160*	0.222*	0.029	0.987	-0.64
Sphenicidae	**42**	*Aptenodytes patagonicus*	king penguin	LC	6.2	4	64	S	3.90	CR	615	0.017	0.018	0.022	0.996	1.42
Sphenicidae	**43**	*Eudyptes antipodes*	yellow-eyed penguin$^{\Psi}$	EN	3.17	7	350	S	2.90	CR	731	0.220*	0.222*	0.002	0.501	-0.13
Sphenicidae	**44**	*Eudyptes chrysocome*	rockhopper penguin$^{\delta}$	VU	6.39	4	20	S	3.30	Cytb	668	0.946*	0.911*	0.007	0.784	0.88
Sphenicidae	**45**	*Eudyptula minor*	little penguin	LC	5.69	16	477	S	3.70	CR	387	0.815*	0.823*	0.071	0.959	3.29**
Sphenicidae	**46**	*Pygoscelis adeliae*	adélie penguin	NT	6.69	15	528	S	3.60	CR	594	-	0.110	0.043	0.998	-1.71*
Sphenicidae	**47**	*Pygoscelis antarcticus*	chinstrap penguin	LC	6.77	4	166	S	3.30	CR	441	0.028	0.027	0.007	0.963	-1.87*
Sphenicidae	**48**	*Pygoscelis papua*	gentoo penguin	LC	5.6	6	249	S	3.18	CR	307	0.577*	0.610*	0.022	0.977	-0.88
Sphenicidae	**49**	*Spheniscus magellanicus*	magellanic penguin	NT	6.17	9	87	S	3.00	COI	686	0.050	0.048	0.002	0.687	-2.25**
Laridae	**50**	*Larus argentatus*	European herring gull$^{\delta}$	LC	6.00	11	131	N	3.70	CR	389	0.215*	0.316*	0.012	0.888	-0.60
Laridae	**51**	*Larus cachinnans*	yellow-legged gull$^{\delta}$	LC	5.69	24	433	N	3.95	CR	431	0.725*	0.722*	0.017	0.937	0.86
Laridae	**52**	*Larus canus*	common gull	LC	6.17	5	60	N	3.78	CR	288	0.416*	0.410*	0.025	0.976	-0.25
Laridae	**53**	*Larus fuscus*	lesser black-backed gull	LC	5.69	21	272	N	3.60	CR	430	0.183	0.192	0.003	0.746	-2.03*
Laridae	**54**	*Larus glaucescens*	glaucous-winged gull	LC	5.39	7	43	N	3.30	CR	386	0.125	0.103	0.016	0.954	-0.36
Laridae	**55**	*Larus hyperboreus*	glaucous gull	LC	5.87	5	61	N	3.48	CR	392	0.697*	0.744*	0.016	0.921	-0.45
Laridae	**56**	*Larus marinus*	great black-backed gull	LC	5.07	14	74	N	3.70	CR	391	0.196*	0.247*	0.011	0.891	0.02
Laridae	**57**	*Rissa brevirostris*	red-legged kittiwake	VU	5.3	3	27	N	3.18	CR	445	0.176*	0.176*	0.211	0.915	-1.01

Laridae	**58**	*Gygis alba*	white tern	LC	5.00	7	209	T	3.90	Cytb	502	0.440*	0.351*	0.004	0.716	-1.22
Laridae	**59**	*Sterna hirundinacea*	American tern	LC	4.87	6	151	S	3.48	NADH	799	-0.013	-0.008	0.001	0.714	-0.23
Laridae	**60**	*Gelochelidon nilotica*	gull-billed tern	LC	5.30	3	44	N	3.60	Cytb	719	0.114	0.129	0.003	0.890	-0.90
Laridae	**61**	*Sterna antillarum*	least tern	LC	4.92	20	188	N	3.60	CR	840	0.138	0.084	0.005	0.968	-1.43
Laridae	**62**	*Sterna fuscata*	sooty tern$^{\Psi}$	LC	7.00	5	56	T	4.18	CR	343	0.356*	0.338*	0.027	0.993	-0.71
Alcid	**63**	*Aethia cristatella*	crested auklet	LC	6.60	5	75	N	3.18	CR	406	0.020	0.019	0.013	0.996	-1.74*
Alcid	**64**	*Aethia pygmaea*	whiskered auklet	LC	4.69	4	59	N	3.40	CR	671	0.131*	0.138*	0.006	0.989	-1.73*
Alcid	**65**	*Alca torda*	razorbill	NT	5.69	5	123	N	3.70	CR	300	0.034	0.042	0.012	0.918	-1.48*
Alcid	**66**	*Alle alle*	little auk	LC	7.56	3	75	N	3.30	CR	481	-0.013	-0.012	0.008	0.979	-1.60*
Alcid	**67**	*Brachyramphus brevirostris*	kittlitz's murrelet	NT	4.47	3	48	N	3.48	CR	330	0.935*	0.941*	0.018	0.777	1.59
Alcid	**68**	*Brachyramphus marmoratus*	marbled murrelet$^{\delta}$	EN	5.30	9	47	N	3.70	Cytb	1044	0.981*	0.972*	0.016	0.573	-1.14
Alcid	**69**	*Ptychoramphus aleuticus*	cassin's auklet	NT	6.25	18	223	N	3.80	CR	649	0.304*	0.307*	0.008	0.948	-0.65
Alcid	**70**	*Synthliboramphus antiquus*	ancient murrelet	LC	6.00	4	58	N	3.70	CR	1131	0.005	0.006	0.004	0.845	1.39
Alcid	**71**	*Synthliboramphus hypoleucus*	xantus's murrelet$^{\delta}$	LC	3.95	13	443	N	2.90	CR	409	0.692*	0.698*	0.010	0.820	-1.15
Alcid	**72**	*Uria aalge*	common guillemot	LC	7.00	29	575	N	3.85	CR	699	0.614*	0.647*	0.009	0.891	-1.74*
Alcid	**73**	*Uria lomvia*	brünnich's guillemot	LC	7.00	19	457	N	3.81	CR	742	0.428*	0.430*	0.013	0.951	-1.20*

[a]VU: Vulnerable, CR: Critically Endangered, LC: Least Concern, NT: Near Threatened, EN: Endangered.

[b]Population size in breeding pairs expressed in Log_{10}.

[c]N: Northern Temperate species, S: Southern Temperate species, T: Tropical species, NS: species sampled in the Northern and the Southern Hemisphere out of the Tropical zone (see definition in section Material and Method).

[d]Maximum marine distance among colonies expressed in Log_{10}.

[e,f]F-statistics: * $p<0.05$.

[g]Tajima's D: * $p<0.05$ ** $p<0.01$.

[h]Restriction Enzymes.

$^{\delta}$species showing distinct colonies currently elevated to full species status as reported in HWB and BirdLife Taxonomic Checklist 2017. See Supplementary information SI. 1

$^{\Psi}$Species whose the scientific names have been revised as reported in HWB and BirdLife Taxonomic 2017. See Supplementary Information SI. 1

1 Lombal *et al*., 2018; **2** Genovart *et al*., 2007; **3** Austin *et al*. , 1994; **4** Gómez-Díaz *et al*., 2009; **5** Burg and Croxall, 2004; **6** Burg *et al*., 2003; **7** Quillfeldt *et al*., 2017; **8** Cagnon *et al*., 2004; **9** Techow *et al*., 2010; **10** Techow *et al*., 2010; **11** Smith and Friesen, 2007; **12** Bicknell *et al*., 2012; **13** Quillfeldt *et al*., 2017; **14** Quillfeldt *et al*., 2017; **15** Ovenden *et al*., 1991; **16** Silva *et al*., 2015; **17** Young, 2010; **18** Walsh and Edwards, 2005; **19** Techow *et al*., 2009; **20** Brown *et al*., 2010; **21** Rayner *et al*., 2011; **22** Gangloff *et al*., 2013; **23** Welch *et al*., 2011; **24** Wiley *et al*., 2012; **25** Lombal *et al*., 2016; **26** Abbott and Double, 2003; **27** Burg and Croxall, 2001; **28** Burg and Croxall, 2001; **29** Abbott and Double, 2003; **30** Steeves *et al*., 2003; **31** Levin and Parker, 2012; **32** Morris-Pocock *et al*., 2010; **33** Taylor *et al*., 2011; **34** Morris-Pocock *et al*., 2010; **35** Taylor *et al*., 2011; **36** Barlow *et al*., 2011; **37** Calderón *et al*., 2014; **38** Mercer *et al*., 2013; **39** Marion and Le Gentil, 2006; **40** Calderón *et al*., 2014; **41** Younger *et al*., 2015; **42** Clucas *et al*., 2016; **43** Boessenkool *et al*., 2009; **44** Banks *et al*., 2006; **45** Grosser *et al*., 2015; **46** Ritchie *et al*., 2004; **47** Clucas *et al*., 2014; **48** Clucas *et al*., 2014; **49** Bouzat *et al*., 2009; **50** Sonsthagen *et al*., 2012; **51** Liebers *et al*., 2001; **52** Sonsthagen *et al*., 2012; **53** Liebers and Helbig, 2002; **54** Sonsthagen *et al*., 2012; **55** Sonsthagen *et al*., 2012; **56** Pons *et al*., 2013; **57** Patirana *et al*., 2002; **58** Yeung *et al*., 2009; **59** Faria *et al*., 2010; **60** Miller *et al*., 2013; **61** Draheim *et al*., 2010; **62** Avise *et al*., 2000; **63** Pshenichnikova *et al*., 2015; **64** Pshenichnikova *et al*., 2017; **65** Moum and Arnason, 2001; **66** Wojczulanis-Jakubas *et al*., 2015; **67** Birt *et al*., 2011; **68** Friesen *et al*., 1996; **69** Wallace *et al*., 2014; **70** Pearce *et al*., 2002; **71** Birt *et al*., 2011; **72** Morris-Pocock *et al*., 2008; **73** Tigano *et al*., 2015.

Mitochondrial DNA sequences (Cytochrome b, Control Region (CR), COX1, NADH or ATPase) available in GenBank were combined using published haplotype frequencies for each sampled colony within species for a total of 68 species (GenBank accession numbers available in Supplementary Information SI. 6 – 2). In total, estimates of Tajima's D were obtained for 70 species (Table 6 – 1). Information on migratory status, non-breeding distributions for migratory species, and differences in morphology and breeding phenology among colonies were obtained for 73, 20, 48 and 33 species, respectively (Supplementary Information SI. 6 – 3). For Northern Temperate and Tropical species, occurrence and location of land separating sample colonies is reported in Supplementary Information SI. 6 – 4, 5. For Southern Temperate species, the presence of sample colonies on islands identified as refuge zones is reported in Supplementary Information SI. 6 – 6. Localities of non-breeding areas were collected and mapped for 13 migratory species exhibiting different post-breeding areas among colonies (Supplementary Information SI. 6 – 7). Triangulations based on the Monmonier's algorithm were built for the 49 species showing a significant F_{st} (except for the short-tailed shearwater and the great cormorant for which we could not build a pairwise F_{st} matrix and for the emperor penguin exhibiting an entire sampling range in Antarctica; scientific names in Table 1), representing a total of 351 colonies and six seabird families (Figure 1). Non-breeding distributions for 13 species were derived from analysis of published telemetry data and represented on a world map (Supplementary Information SI. 7).

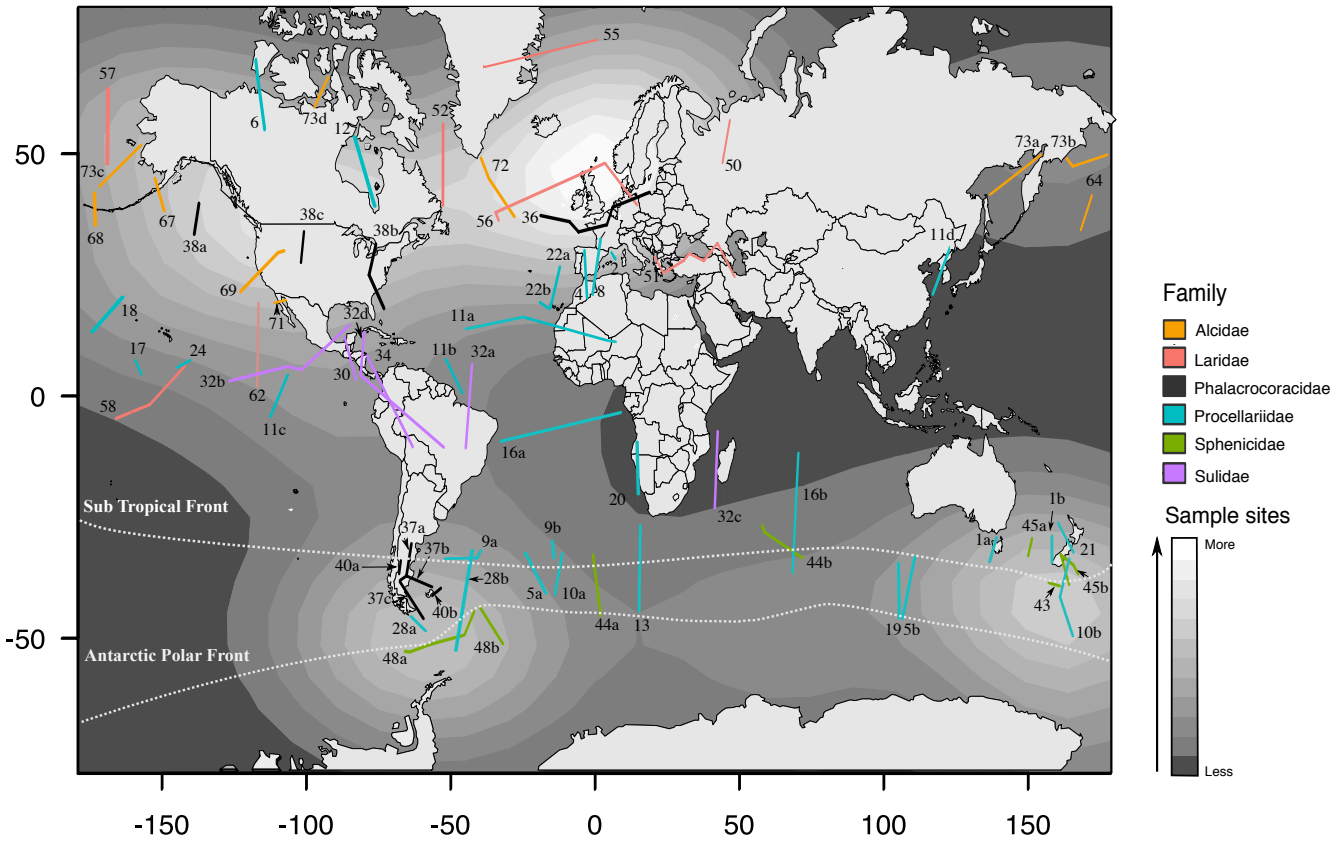

Figure 6 – 1 Triangulations based on the Monmonier's algorithm built for the 49 seabird species showing a significant F_{st} representing a total of 351 colonies and six seabird families. The Monmonier's maximum distance algorithm identifies the areas where F_{st} shows an abrupt rate of change. Each line represents an approximation of the highest probability of localization of single or multiple barriers to dispersal within species. Letter 'a' on map represents the highest F_{st} within colonies of each species when multiple barriers to dispersal were detected. Density of grey represents the accumulated number of breeding sites sampled in the overall multi-species analyses. **Procellariidae: 1 (a–b)** flesh-footed shearwater, **2** Balearic shearwater, **4** Cory's shearwater, **5 (a–b)** wandering Albatross, **6** northern fulmar, **8** European storm petrel, **9 (a–b)** southern giant petrel, **10 (a–b)** northern giant petrel, **11 (a–c)** band-rumped storm petrel, hot versus cool season breeders are not indicated on map, **12** Leach's storm petrel, **13** thin-billed prion, **16 (a–b)** white-faced storm

petrel, **17** Laysan albatross, **18** black-footed albatross, **19** white chinned petrel, **20** Trinidade petrel, **21** Cook's petrel, **22 (a–b)** gadfly petrel, **24** hawaiian petrel, **28 (a–b)** black-browed albatross. **Sulidae: 30** masked booby, **32 (a–d)** brown booby, **34** red-footed booby. **Phalacrocoracidae**: **36** European cormorant, **37 (a–c)** imperial shag, **38 (a–c)** double-crested cormorant, **40 (a–b)** rock shag. **Sphenicidae: 43** yellow-eyed penguin, **44 (a–b)** rockhopper penguin, **45 (a–b)** little penguin, **48 (a–b)** gentoo penguin. **Laridae**, **50 (a–c)** European herring-gull**, 51** yellow-legged gull**, 52** common gull, **55** glaucous gull, **56** great black-backed gull, **57** red-legged kittiwake, **58** white tern, **62** sooty tern. **Alcid**: **64 (a–b)** whiskered auklet, **67** kittlitz's murrelet, **68** marbled murrelet, **69** cassin's auklet, **71** xantus's murrelet, **72 (a–c)** common guillemot, **73 (a–d)** brünnich's guillemot. Triangulation could not be conducted for **3** short-tailed shearwater and **39** great cormorant as a pairwise F_{st} matrix for these species could not be built based on the genetic information obtained in the literature and for **46** adélie penguin exhibiting an entire sampling range in Antarctica. Scientific names in Table 6 – 1.

Impact of mutation-drift equilibrium on genetic structure

Among 70 species for which Tajima's D was calculated, 14 species showed a significant values including four species also showing a significant F_{st} (little penguin, whiskered auklet, common murre and brünnich's guillemot; see 45, 64, 72, 73 in Table 6 – 1). *Post hoc* tests conducted for these four species showed a significant D* for at least one group of colonies forming an independent genetic cluster (Supplementary Information SI. 6 – 8, 9, 10, 11) indicating that the observed bottleneck signal for these species cannot reflect significant genetic structuring among colonies.

GLM M1 revealed a significant effect of mutation-drift equilibrium on F_{st} when tested with a normal or binomial error distribution (M1, Table 6 – 2; Table 6 – 3). F_{st} were significantly lower in species showing significant Tajima's D (ANOVA: F = 9.63, d.f. = 68, $p = 0.003$; Figure 6 – 2A). Mutation-drift equilibrium was independent of geographic regions ($\chi^2 = 1.56$, d.f. = 3, $p = 0.67$), but some taxonomic families were more represented than others ($\chi^2 = 13.4$, d.f. = 5, $p = 0.020$). Among 14 species showing significant Tajima's D, ~42% (6/14) belonged to the alcids, ~28% (4/14) were spheniscids, ~ 21% (3/14) were procellariids, and ~8% (1/14) were larids (Figure 6 – 2B); no sulids or Phalacrocoracids were significant. Significant Tajima's D were always negative except for the little penguin, which was the species exhibiting the highest genetic structure ($F_{st} = 0.815$; Figure 6 – 2B; scientific name in Table 6 – 1). Tropical and Southern Temperate species showing significant Tajima's D were spread in all oceans whereas Northern Temperate species were mostly located in the Northeastern Atlantic Ocean and around the Bering Sea (see sampling sites of crested auklet, whiskered auklet, little auk, razorbill, common guillemot and brünnich's guillemot exhibiting significant D* 63 – 66, 72 and 73 in Supplementary Information SI. 6 – 1; scientific names in Table 6 – 1).

Table 6 – 2 Significant generalized linear models M1 (M1') – M3, M5 of genetic structure in seabird species. Global dataset M1 based on i) 70 species for which Tajima's D could be calculated or obtained in the literature and ii) 59 species that are in mutation-drift equilibrium (73 species - 14 species showing significant Tajima's D = 59 species). Additional models based on species in mutation-drift equilibrium and for which information on breeding phenology (M2; 24 species), morphology (M3; 36 species) and breeding phenology and morphology (M5; 22 species) were available. Models testing the impact of migratory status and differences in post-breeding distributions (M4a–c) on genetic structure were not significant and are not shown.

			Variables in specific model		χ^2		AIC	
Degree of freedom	Group of models	Model	F_{st} as binomial error distribution	F_{st} as normal error distribution	Binomial	Normal	Binomial	Normal
Species for which Tajima's D was obtained (d.f. = 70)	Single factor	M1	D***	D***	**0.001**	**0.003**	81.44	185.46
With respect to mutation-drift equilibrium (d.f. = 59)	Single factor	M1'	Sample sites*	Sample sites	**0.040**	0.406	62.04	157.30
With respect to mutation-drift equilibrium (d.f. = 24)	Single factor	M2	Differences in phenology (P)	Differences in phenology (P)*	0.056	**0.024**	29.45	78.94
With respect to mutation-drift equilibrium (d.f. = 36)	Single factor	M3	Differences in morphology (M)*	Differences in morphology (M)	**0.007**	0.058	33.57	97.64
With respect to mutation-drift equilibrium (d.f. = 22)	Single additional factor	M5	Differences in morphology (M) + (P)*	Differences in morphology (M) + (P)*	**0.005**	**0.020**	17.09	66.97
	Multiple additional factors	M5	(M) + (P) + h	(M) + (P)* + h	**0.001**	**0.041**	12.41	66.59

Variables in specific model: ***: $p < 0.001$, **: $p < 0.01$, *: $p < 0.05$; χ^2 : bold: $p < 0.05$; shaded values represent the best AIC within significant models for the overall group of models (e.g. multiple additional factors in comparison to single additional factor in M5).

Table 6 – 3 Recapitulative results of the significant predictors of genetic structure within seabird colonies obtained with GLMs performed on all species M1 (M1') – M4a–c, Northern Temperate species N1 – N4a–c, Tropical species T1 – T4a–c and Southern Temperate species S1 – S4a–c. Predictors of genetic structure that were significant for at least one model (F_{st} implemented as binomial or continuous values) as a single factor are reported. Full results are available in Supporting Information SI. 6 – 12, 14, 15, 16.

	Single predictor					
	Tajima's D	Physical isolation	Phenology	Morphology	Haplotype diversity (h)	Sample sites
All species	S***	-	-	-	-	-
All species in relation to mutation-drift equilibrium	-	-	S*	S*	NS	S*
Northern Temperate species	-	NS (Land)	NS	NS	S**	NS
Tropical species	-	S* (Land)	NS	S**	NS	NS
Southern Temperate species	-	S* (Refuge zones)	NS	S***	NS	S*

S=significant, NS=non-significant, ***: $p < 0.001$, **: $p < 0.01$, *: $p < 0.05$

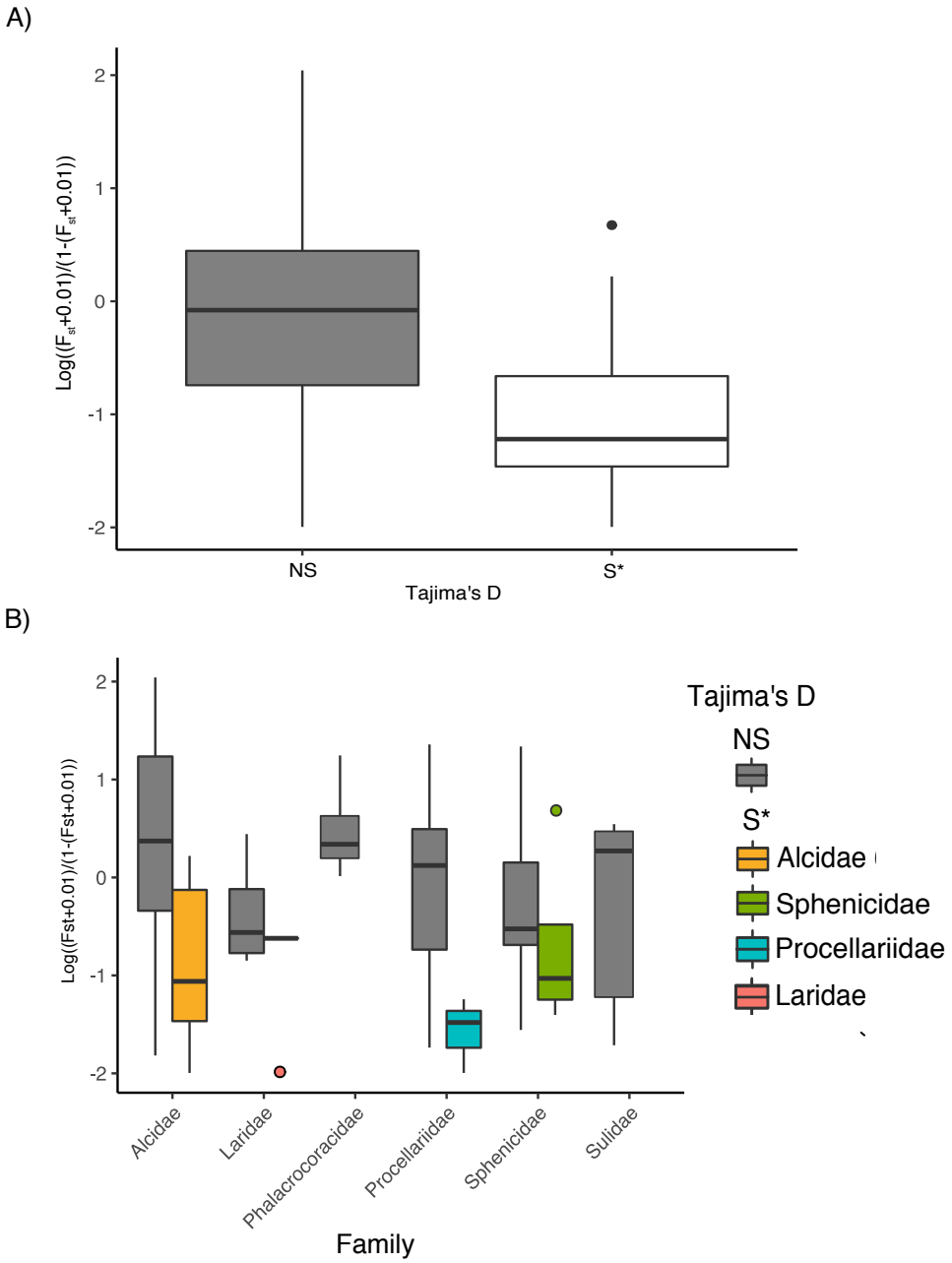

Figure 6 – 2 Mean study-wide F_{st} of seabird species in relation to mutation-drift equilibrium. A) Means of F_{st} across all species for which the Tajima's D test was performed (n = 70) with respect to mutation-drift equilibrium, B) Means of F_{st} across six seabird families. The horizontal lines that form the top of boxes are the 75th percentile (Q1). The horizontal lines that form the bottom of the boxes are the 25th percentile (Q3). The horizontal lines that intersect the boxes are the median F_{st}. Distributions are presented separately for species with respect to whether Tajima's D is significant.

Biotics factors and variation in phenotypical traits as predictors of genetic structure

To discard potential bias of demographic history on genetic structure, the 14 species exhibiting significant Tajima's D were removed from our dataset and GLM models M1'–M4a–c were run with the remaining observations. In M1', when single factors were tested, the lowest AIC was obtained for the number of sample sites when F_{st} was implemented as a binomial factor (M1', Table 6 – 2; Table 6 – 3). However, linear regression failed to detect correlation between F_{st} and the number of sampling sites ($F = 0.873$, d.f. = 59, $p = 0.354$, $R^2 = 0.015$; Supplementary Information SI. 6 – 13). M1' showed no significant differences in genetic structure among Northern Temperate, Tropical or Southern Temperate species (ANOVA: $F = 1.55$, d.f. = 54, $p = 0.221$, AIC for all single factors shown in Supplementary Information SI. 6 – 12). Among M2 – M4a–c, population differences in phenology and morphology were significant as single factors when F_{st} was implemented as a continuous variable and as a binomial factor, respectively (M2, M3, Table 6 – 2; Table 6 – 3). F_{st} differed between species in relation to phenology (ANOVA: $F = 5.96$, d.f. = 24, $p = 0.022$) and morphology (ANOVA: $F = 5.52$, d.f. = 36, $p = 0.024$). One additional model, M5, including population differences in morphology and phenology as additional predictors, was significant and AIC was improved with haplotype diversity as an additional predictor (M5, Table 6 – 2). Genetic marker, taxonomic family, migratory status, variation in non-breeding distributions, marine distance between the most distant colonies, IUCN status, population size, sample size were never significant in any GLM (M1' – M4a–c, Supplementary Information SI. 6 – 12).

Land or ice barriers, refuge zones and variation in phenotypic traits as predictors of genetic structure in relation to regions

In Northern Temperate species, presence of land separating colonies was not a significant predictor of F_{st} (N1, Supplementary Information SI. 6 – 14). F_{st} did not differ depending on whether colonies were fragmented by land (ANOVA: $F = 0.855$, d.f. = 26, $p = 0.364$), and a similar proportion of species showed significant F_{st} (86% (13/15) and 81% (9/11) fragmented or not, respectively; Supplementary Information SI. 6 – 4). F_{st} did not differ between colonies located in Mediterranean vs. Atlantic, Pacific vs. Atlantic, Atlantic vs. Indian Ocean or across the Bering Sea (ANOVA: $F = 0.512$, d.f. = 12, $p = 0.682$). GLM model N1 detected significant effects of haplotype diversity and the number of sample sites when F_{st} was implemented as a continuous value (Table 6 – 3; Supplementary Information SI. 6 – 14). Haplotype diversity and the number of sample sites explained 38% of the model ($F = 7.21$, d.f. = 26, $p = 0.004$, $R^2 = 0.38$; Figure 6 – 3A). The predominant predictor of F_{st} in N1 was haplotype diversity, which was significant as a single factor, explaining 32% of the model ($F = 11.48$, d.f. = 26, $p = 0.002$, $R^2 = 0.32$), where F_{st} decreased as h increased (Figure 6 – 3A).

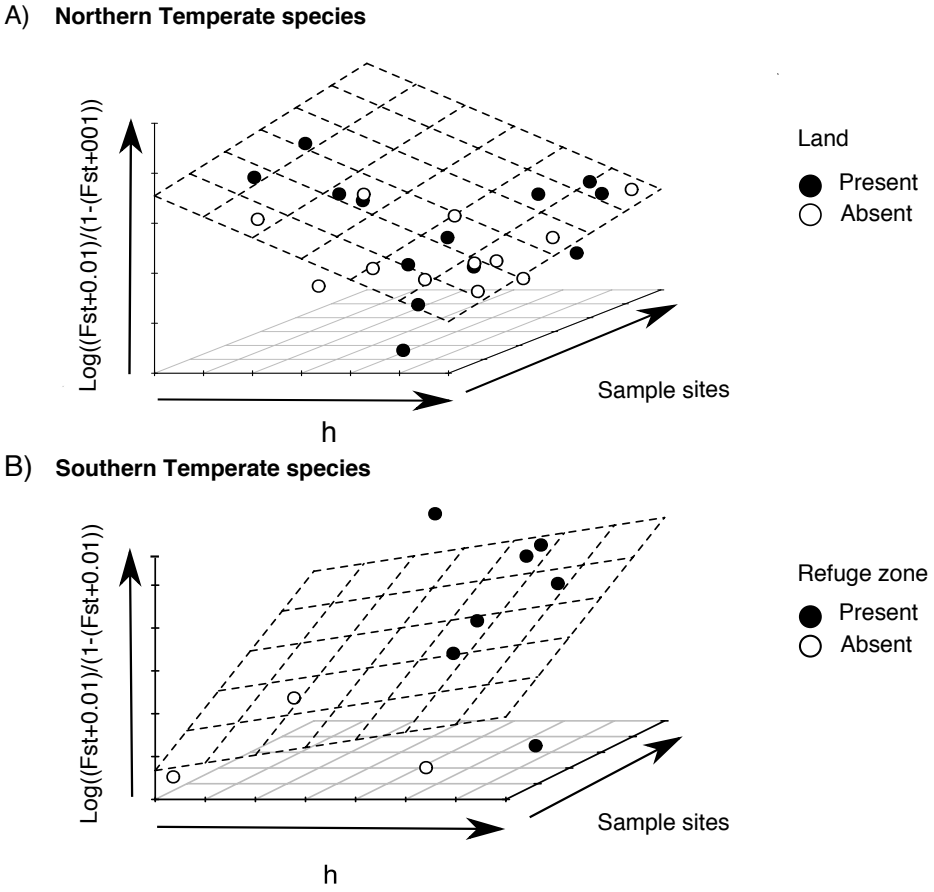

Figure 6 – 3 Regression surface showing the relationship between F_{st} and significant predictors of genetic structure in Northern and Southern Temperate species exhibiting mutation-drift equilibrium. A) In Northern Temperate species (Model N1), the best predictor of F_{st} was haplotype diversity explaining 32% of the model. Presence of land separating colonies was not significant. B) In Southern Temperate species (Model S1), both the number of sample sites and the presence of colonies in refuge zones were the best predictors of F_{st} explaining 39% and 38% of the model, respectively.

In Northern Temperate species, neither differences in phenology (N2), morphology (N3), migratory status or variation in non-breeding distributions among colonies in migratory species (N4a–c) were significant predictors of F_{st} (N2 – N4a–c, Supplementary Information SI. 6 – 14). However, most species exhibiting morphological differences across their sampling range showed a significant F_{st} (88%; 14/16; Figure 6 – 4A). The only two species exhibiting morphological variation across their breeding range and showing a non-significant F_{st} (60 – 61 in Figure 6 – 4A) also exhibited a negative Tajima's D (gull-billed tern $F_{st} = 0.114$, D = -0.904; least tern $F_{st} = 0.138$, D = -1.43; 60, 61 in Table 6 – 1). All species showing variation in breeding phenology showed a significant F_{st} (Figure 6 – 4A). Variation in morphology and phenology appear in comparable proportions in relation to the presence of land across the sampling range within species (morphology: absence of land = 100% (5/5), presence of land = 82% (9/11); phenology: absence of land: 100% (3/3), presence of land: 75% (3/4); Figure 6 – 4A). A total of six species showed variation in both morphology and breeding phenology across their sampling range including three species whose colonies were not separated by land (gadfly petrel, cassin's auklet, xantus's murrelet; 22, 69, 71 in Figure 6 – 4A; scientific names in Table 6 – 1).

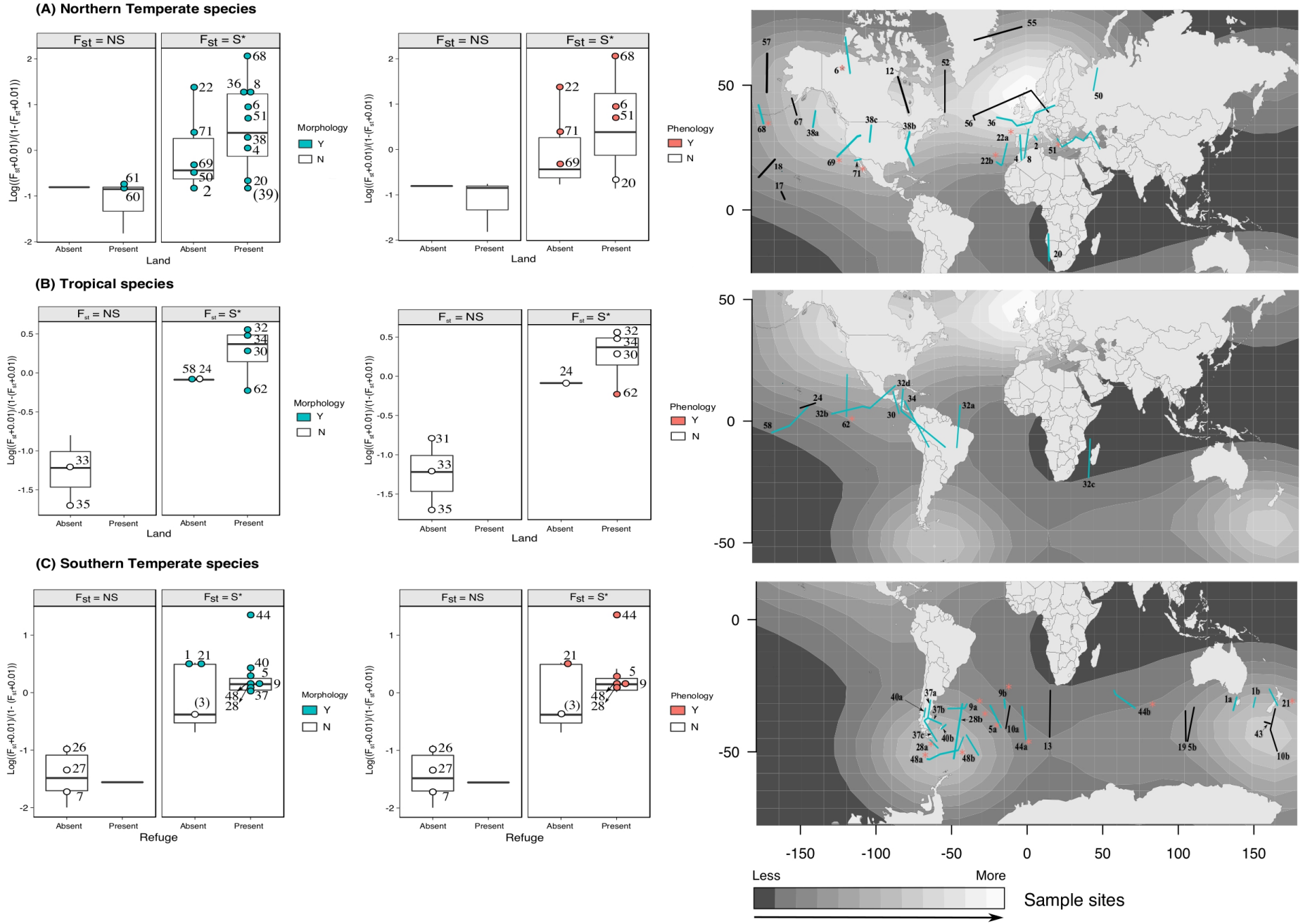
(A) Northern Temperate species
(B) Tropical species
(C) Southern Temperate species
Fst = NS
Fst = S*
Log((Fst+0.01)/(1-(Fst+0.01)))
Absent
Present
Land
Refuge
Morphology
Phenology
Y
N
Less
More
Sample sites
-150
-100
-50
0
50
100
150

Figure 6 – 4 Mean study-wide F_{st} of Northern Temperate, Tropical and Southern Temperate species in relation to historical fragmentation, morphological and phenological variation. All 59 species showing mutation-drift equilibrium are represented in the boxplots A – C but only the species indicated with numbers in the boxplots are the species for which information on differences in morphology and breeding phenology among populations were available in the literature. On the maps, only species exhibiting a significant F_{st} among colonies are shown. Blue lines represent the species showing differences in morphology among colonies. Pink stars represent species showing variation in breeding phenology across their sampling range in addition to variation in morphology. Black lines represent species showing a significant F_{st} for which information on morphology was not available in the literature; except for the n°24 that does not show variation in morphology. See Table 6 – 1; Figure 6 – 1 for the species numbers. () = species not shown on map for which the triangulation could not be conducted. Density of grey represents the accumulated number of sample sites sampled in the overall multi-species analyses. The wandering albatross 5 only showed morphological variation among colonies separated by the barrier 5a as indicated on map 4C.

In tropical species, the presence of sample colonies across the Isthmus of Panama appears as significant predictor of genetic structure when F_{st} was implemented as a binomial factor (T1, Table 6 – 3; Supplementary information SI. 6 – 15). This predictor explained 50% of genetic structure ($F = 6.93$, d.f. = 9, $p = 0.033$, $R^2 = 0.50$). Among models T2 – T3, only differences in morphology (T3) was significant as a single predictor of genetic differentiation (Table 6 – 3; T3, Supplementary Information SI. 6 – 15). Variation in morphological traits explained 61% of the model ($F = 8.45$, d.f. = 8, $p = 0.027$, $R^2 = 0.61$). All tropical species showing morphological differences across their sampling range showed a significant F_{st} (5/5, Figure 6

– 4B) and 80% (4/5, Figure 6 – 4B) of these species showed a sampling range fragmented by the Isthmus of Panama. As insufficient information on migratory status and variation in non-breeding distributions was available for Tropical species (most Tropical species are year-resident) and given that 88% (8/9) tropical species are classified as Least Concern (Table 6 – 1), T4a–c and IUCN status could not be tested.

In Southern Temperate species, the presence of sample colonies in refuge zones was a significant predictor of population differentiation (Table 6 – 3; S1, Supplementary Information SI. 6 – 16). Sister-species comparisons highlight this difference. For example, the black-browed albatross was sampled in the Falkland Islands and showed a significant F_{st} (28 in Table 6 – 1; Figure 6 – 4C; see sampling range in Supplementary Information SI. 6 – 1), whereas the grey-headed albatross, with a similar sampling range except the Falkland Islands, showed a very low F_{st} (27 in Table 6 – 1; see sampling range in Supplementary Information SI. 6 – 1). A similar pattern was observed for the blue petrel, which is absent from refuge zones and showed a low F_{st} (7 in Table 6 – 1, see sampling range in Supplementary Information SI.6 – 1), compared to the thin-billed prion, which was sampled on the Falkland Islands and showed a significant F_{st} (13 in Table 6 – 1; Figure 6 – 4C; see sampling range in Supplementary Information SI. 6 – 1). Moreover, four out of the five species that were not sampled in refuge zones but exhibited significant F_{st} were located close to New Zealand, a region that maintained ice-free areas (flesh-footed shearwater, Cook's petrel, short-tailed shearwater and yellow-eyed penguin; see 1, 21, 43 on map of Figure 6 – 4C and 3 in Supplementary Information SI. 6 – 1; scientific names in Table 6 – 1).

Hierarchy of genetic partitioning among Southern Temperate species

Among Southern Temperate species sampled in refuge zones, all species that were sampled on the Falkland Islands showed their highest genetic partitioning around that zone, including southern giant petrels and rockhopper penguins, whose sampling range included northern islands (Gough Island and Amsterdam Island, respectively; Figure 6 – 5; scientific names in Table 6 – 1). Northern giant petrels (scientific name in Table 6 – 1), which were not sampled on the Falkland Islands but that were sampled in South Georgia, also showed the highest genetic partitioning among colonies in that region, but genetic divergence was lower than that of the southern giant petrels (F_{st} = 0.207 vs. 0.572; Figure 6 – 5). However, of the five species that were sampled on South Georgia (glaciated during the Pleistocene) but not on the Falkland Islands — wandering albatross, blue petrel, northern giant petrel, white-chinned petrel and grey-headed albatross (5, 7, 10, 19, 27 in Supplementary Information SI. 6 – 1; scientific names in Table 6 – 1) — northern giant petrel was the only species showing significant F_{st}.

Number of sampling sites and phenotypic traits as predictors of genetic structure among Southern Temperate species

The lowest AIC in model S1 was obtained for number of sample sites, IUCN status, haplotype diversity, and the presence of sampled colonies in refuge zones (S1, Supplementary Information SI. 6 – 16), explaining 75% of the variation in F_{st} ($F = 7.23$, d.f. = 22, $p = 0.001$, $R^2 = 0.75$). However, significant variation in the number of species in different categories of IUCN status was suspected to introduce a bias in the linear regression (Table 6 – 1; Supplementary Information SI. 6 – 17). Hence, a multiple regression including the number of sample sites, the presence of sampled colonies in refuge zones and haplotype diversity was performed on species classified as Least Concern only, as this category represents 50% of

Southern Temperate species studied. These three predictors explained 64% of the variation ($F = 3.71$, d.f. = 6, $p = 0.08$, $R^2 = 0.64$; Figure 6 – 3B). The two major predictors of F_{st} were the number of sampling sites, explaining 39% of the model ($F = 5.9$, d.f. = 9, $p = 0.038$, $R^2 = 0.39$; Figure 6 – 3B), and the presence of sample colonies in refuge zones, explaining 38% of the model ($F = 5.49$, d.f. = 9, $p = 0.044$, $R^2 = 0.38$; Figure 6 – 3B).

Number of sampling sites and phenotypic traits as predictors of genetic structure among Southern Temperate species

The lowest AIC in model S1 was obtained for number of sample sites, IUCN Status and haplotype diversity in addition to the presence of sampled colonies in refuge zones as predictors of genetic structure (S1, Supplementary Information SI. 6 – 16). These four predictors explained 75% of the variation in population genetic structure ($F = 7.23$, d.f. = 22, $p = 0.001$, $R^2 = 0.75$). However, significant variation in the number of observations in different categories of IUCN status was suspected to introduce a bias in the linear regression (Table 6 – 1; Supplementary Information SI. 6 – 17). Hence, a multiple regression including the number of sample sites, the presence of sampled colonies in refuge zones and haplotype diversity was performed on species classified as Least Concern only, as this category represents 50% of Southern Temperate species studied. These three predictors explained 64% of the variation ($F = 3.71$, d.f. = 6, $p = 0.08$, $R^2 = 0.64$; Figure 6 – 3B). The two major predictors of F_{st} were the number of sampling sites, explaining 39% of the model ($F = 5.9$, d.f. = 9, $p = 0.038$, $R^2 = 0.39$; Figure 6 – 3B), and the presence of sample colonies in refuge zones, explaining 38% of the model ($F = 5.49$, d.f. = 9, $p = 0.044$, $R^2 = 0.38$; Figure 6 – 3B). These two factors were the predominant predictors of F_{st}, explaining 59% of the model ($F = 5.75$, d.f. = 9, $p = 0.028$, $R^2 = 0.59$).

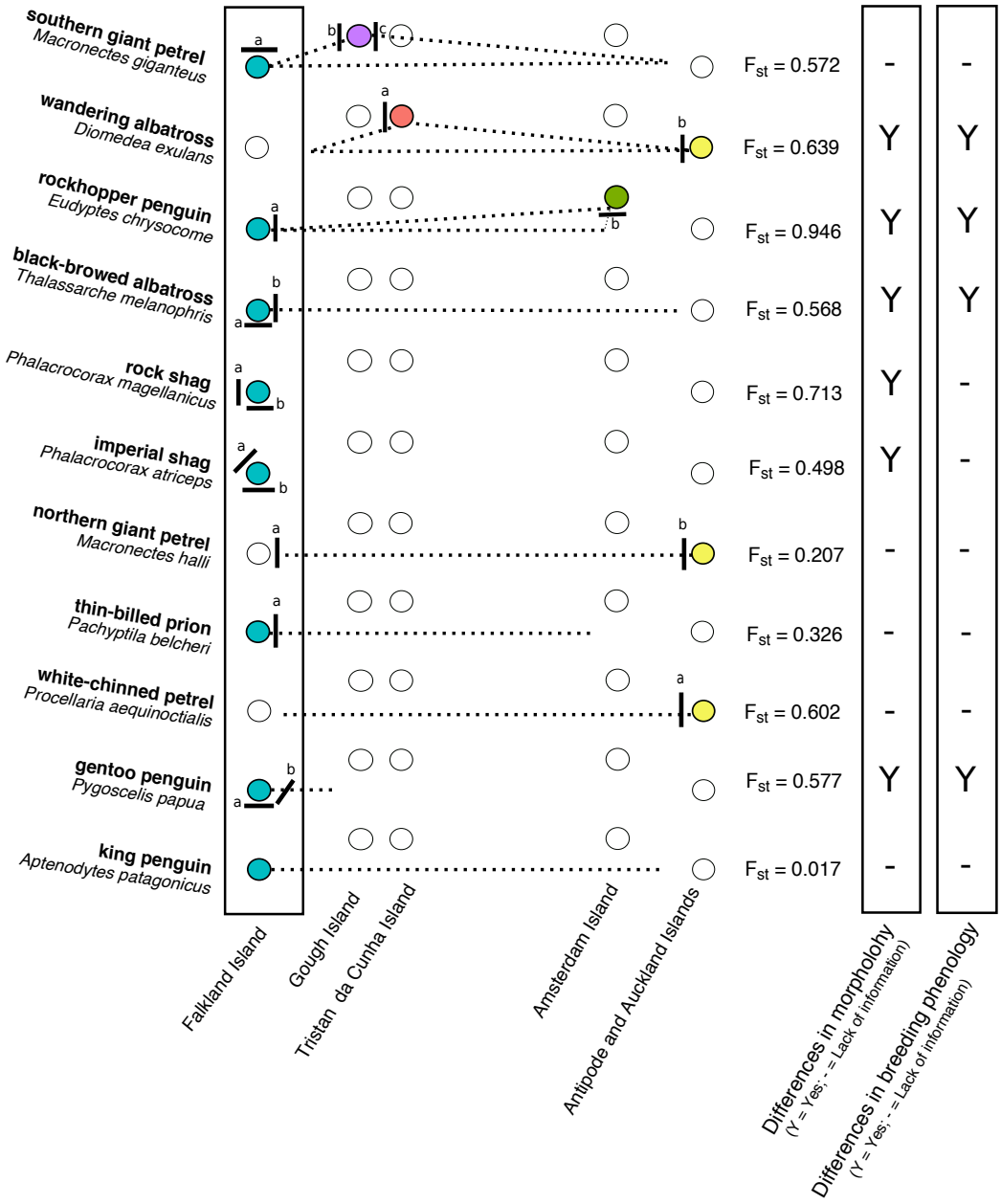

Figure 6 – 5 Multiple level comparison of F_{st} in Southern Temperate species showing sample colonies in refuge zones. All species that were sampled on the Falkland Islands exhibited their major genetic partitioning between colonies in this region and the other sample colonies. Refuge zones are located on the figure from west to east. Colors indicate presence of colonies sampled in the different refuge zones. Solid lines represent hierarchical barriers to gene flow among colonies (a, b, c). The position of the barriers are related to the sampling range of species (Supplementary Information SI. 6 – 1). Dotted lines represent the sampling range of species.

Among models S2 – S4a–c, only differences in morphology (S3) was significant as a single factor (Table 6 – 3; S3, Supplementary Information SI. 6 – 16). However, S3 showed the lowest AIC when morphology was combined with haplotype diversity alone, or with haplotype diversity and the presence of sample colonies in refuge zones, when F_{st} was implemented as a continuous factor or as a binomial factor, respectively (S3, Supplementary Information SI. 6 – 16). Overall, those three predictors explained 74% of the variation ($F = 8.373$, d.f. = 14, $p = 0.006$, $R^2 = 0.74$). F_{st} differed between species in relation to morphological variation (ANOVA: $F = 23.23$, d.f. = 14, $p = 0.0005$), where all species showing differences in morphology exhibited a significant F_{st} (Figure 6 – 4C). Variation in morphology appears in different proportions in relation to the presence of colonies in refuge zones (absent = 33% (2/6), present = 100% (7/7) respectively; Figure 6 – 4C). Overall, species showing differences in morphology were either sampled in refuge zones or located in New Zealand or around the Tasman Sea (flesh-footed shearwaters, and Cook's petrel; 1, 21 on map of Figure 6 – 4C; scientific names in Table 6 – 1). In model S2, variation in breeding phenology was not significant as a single factor (S2, Supplementary Information SI. 6 – 16), and F_{st} did not differ with respect to phenology (ANOVA: $F = 3.349$, d.f. = 9, $p = 0.1$). Overall, 100% (6/6) of species that showed differences in breeding phenology across their sampling range were either sampled in refuge zones or located in New Zealand (Figure 6 – 4C).

Species showing a sampling range in the Northern and the Southern Hemisphere

Only two seabird species were sampled in both the Northern and the Southern Hemispheres. They both showed high F_{st} (band-rumped storm petrel $F_{st} = 0.730$ and white faced storm petrel $F_{st} = 0.893$; 11, 16 in Table 6 – 1 and Figure 6 – 1; scientific names in Table 6 – 1), and both exhibited colony differences in breeding phenology (Supporting Information SI. 6 – 3).

Neither species showed colony differences in morphology, and no information was available on their non-breeding distributions (Supporting Information SI. 6 – 3).

Impact of migratory status and differences in non-breeding distributions in migratory species on genetic structuring among colonies

Migratory status and variation in non-breeding distributions among colonies in migratory species were never significant in any GLM (models M4a–c, N4a–c, S4a–c in Supplementary Information SI. 6 – 12, 14, 15, 16). In Northern Temperate species, 66% (4/6) of migratory or partially migratory species showing colony differences in non-breeding distributions exhibited significant F_{st} (Cory's shearwater, northern fulmar, double-crested cormorant, and gadfly petrel; see 4, 6, 22, 38 in Table 6 – 1 and Supplementary Information SI. 6 – 7; scientific names in Table 6 – 1). However, 75% (3/4) of those species (see the list above except the gadfly petrel) exhibited a sampling range fragmented by land (4, 6, 38 in Figure 6 – 4A). Similarly, in Southern Temperate species, 75% (3/4) of migratory species showing different non-breeding distributions among colonies exhibited significant F_{st} (black-browed albatross, rockhopper penguins, Cook's petrel; see 28, 44, 21 in Table 6 – 1 and Supplementary Information SI. 6 – 7; scientific names in Table 6 – 1). However, all of those species were either sampled in refuge zones such as the Falkland Islands (black-browed albatross and rockhopper penguins, see 28 and 44 in Figure 6 – 4C; scientific names in Table 6 – 1) or across New Zealand (Cook's petrel; see 21 in Figure 6 – 4C). Moreover, 60% (3/5) of southern species lacking different non-breeding distributions among colonies exhibited a significant F_{st} (flesh-footed shearwater, short-tailed shearwater, thin-billed prion; see 1, 3, 13 in Table 6 – 1; Supplementary Information SI. 6 – 7; scientific names in Table 6 – 1). Large differences in F_{st} were observed among species that were similar with respect to sampling

range and the lack of distinct non-breeding distributions among colonies, but differed in their sampling from refuge zones (e.g. 0.008, blue petrel, no refuge, vs. 0.326 thin-billed prion, refuge; see 7 and 13 in Supplementary Information SI. 6 – 1; scientific names in Table 6 – 1).

Discussion

In this study, I evaluated a candidate set of generalized linear models (GLMs) to identify predictors of population differentiation in mtDNA for 73 seabird species. Lack of mutation-drift equilibrium observed in 19% of species coincided with lower estimates of genetic structure, suggesting strong historical legacies. Presence of land across the sampling range of species, or sampling of breeding colonies representing ice-free Pleistocene refuge zones, were the best predictors of genetic differentiation within Tropical and Southern Temperate species, respectively, and were supported by phenotypic variation. Conversely, most of the other biotic factors such as variation in non-breeding distributions among colonies, population size, IUCN status and higher taxon were not significant predictors of population genetic differentiation.

Impact of mutation-drift equilibrium on genetic structure

Deviation from mutation drift equilibrium is widespread in seabird species (19%) and accompanied with significantly lower F_{st}, suggesting underestimation of genetic structure. Locations of species lacking mutation-drift equilibrium differed according to latitude; Tropical and Southern Temperate species were located throughout the Indo-Atlantic Ocean, whereas Northern Temperate species were mostly located in the Atlantic and around the Bering Sea. This is consistent with localities that have been hypothesized as Pleistocene refugia in other Arctic vertebrates such as the southern edge of the Bering Land Bridge, Newfoundland Bank and Spitsbergen Bank (Avise, 2000), as supported by the observation of higher genetic diversity in several seabird colonies sampled in those regions (e.g. Moum and

Arnason, 2001; Sonsthagen *et al*., 2012). For example, despite a greater number of Razorbill sample colonies in the east Atlantic (65 in Supplementary Information SI. 6 – 1; scientific name in Table 6 – 1), genetic diversity is considerably higher in the west Atlantic, and is represented by a star phylogeny—consistent with expansion from a refugium in this region (e.g. Newfoundland – Moum and Arnason, 2001). Conversely, in Southern Temperate species, both significant demographic changes and colonization of new northern breeding areas after the LGM may explain negative Tajima's D statistics (Younger *et al*., 2016). For example, Clucas *et al*., (2014) detected a dramatic increase in Chinstrap penguin (scientific name in Table 6 – 1) abundance during the Holocene, corresponding to the colonization of new breeding habitat as it became available during deglaciation.

Although Tajima's D neutrality test can be affected by several factors such as substitutional rate heterogeneity and population subdivision, a negative value is a relatively robust predictor of demographic changes such as population expansions or bottlenecks (Ramírez-Soriano *et al*., 2008). However, several additional species showed a non-significant negative Tajima's D values suggesting that the influence of this predictor on F_{st} in our multi-species analyses may be underestimated.

Patterns of genetic differentiation among seabird species in relation to geographic regions

Although F_{st} did not show significant variation among seabird colonies according to geographic regions, different factors appeared to explain F_{st} in each region. In Northern Temperate species, there was a strong negative correlation between F_{st} and haplotype diversity, which may reflect historical demographics. Expanding populations typically show an excess of haplotype diversity (Avise, 2000), and even though species showing a significant signal of demographic changes have been discarded from our analyses, extensive

rapid continued expansions of populations from refugia during the Pleistocene in Northern Temperate species may explain the F_{st} – haplotype diversity correlation (Avise, 2004). Conversely, these relationships may reflect sampling bias. The maximum possible value of F_{st} is constrained by genetic diversity within populations, such that species with higher diversity at the molecular level (heterozygosity or haplotype diversity) will exhibit lower F_{st} (Hedrick, 2004).

Presence of a land or ice barrier failed to predict genetic structure in Northern Temperate species, consistent with Friesen (2015), apparently because high F_{st} are observed for a large proportion of seabird species lacking physical barriers to gene flow. For example, cassin's auklet breeds along the Pacific coast of North America from the Aleutian Islands to Baja California and showed genetic and phenotypic differentiation between northern and southern colonies (Wallace *et al*., 2014; scientific name in Table 6 – 1), associated with pronounced differences in foraging environments (Wolf *et al*., 2009). Therefore, morphological specialization for foraging or the influence of latitude on phenology in addition to the presence of land itself, could explain genetic structure among colonies, as has been observed in other taxa (Kelly and Eernisse, 2007; Salisbury *et al*., 2012). However, we did not test 'latitudinal distribution' as a potential predictor of genetic structure in the present study; this relationship should be further evaluated.

In tropical species, high F_{st} was accompanied by land barriers between sampled colonies (the Isthmus of Panama) and morphological variation, which is consistent with previous studies (Friesen *et al*., 2007; Steeves *et al*., 2005). Although the Isthmus of Panama is only 30 km at its narrowest, its interior is dominated by rugged mountains and upland plains, which may restrict seabirds such as sulids from crossing (Steeves *et al*., 2005).

In Southern Temperate species, the presence of sampled colonies in refuge zones such as northern islands (e.g. Gough Island, Amsterdam Island), the Falkland Islands and New Zealand Sub-Antarctic islands was a strong predictor of F_{st}, and was often accompanied by differences in morphology and phenology. Changes in physical landscape in this region during the Pleistocene can explain differences in genetic structure among colonies (Munro and Burg, 2017)—northern islands remained unglaciated (e.g. Tristan da Cunha, Amsterdam island), whereas islands closer to Antarctica experienced increasing levels of glaciation (Fraser *et al*., 2012), although the Falkland Islands had little ice cover (Hall, 2002). This may explain genetic isolation of colonies sampled on Falkland Islands (Fraser *et al*., 2012). Seven species sampled from the Falkland Islands exhibited the highest genetic structuring in that area, including two species that were sampled on more northern islands: southern Giant petrels and rockhopper penguins on Gough Island and Amsterdam Island, respectively (Figure 6 – 5). The Antarctic Frontal Zone, a relatively mobile climatic boundary (Hall, 1990), has also been proposed as an isolating mechanism for seabird colonies during the last 0.9 Ma (e.g. Burg and Croxall, 2004; de Dinechin *et al*., 2009). Conversely, the West Wind Drift and Antarctic Circumpolar Currents have been implicated for both passive dispersal and recolonization of islands after the LGM in a range of taxa (MacAya and Zuccarello, 2010; Fraser *et al*., 2012), and may explain widespread genetic and phenotypic homogeneity in species such as the white-chinned petrel and the grey-headed albatross (19, 27 in Table 6 – 1; Supplementary Information SI. 6 – 1; scientific names in Table 6 – 1).

While proximity to key foraging areas could be considered an important contributor to the maintenance of seabird colonies through time, and hence the development of genetic divergence, the presence of ice-free refuge zones appears more important. Proximate to the Falkland Islands is the Patagonian shelf, which is of global significance for the diversity and

abundance of top predators (Brown *et al*., 1975; Cooke and Mills, 1972), and also critical for the survival of many seasonal visitors (Croxall and Wood, 2002). However, of the five species that were sampled on South Georgia (glaciated during the Pleistocene) but not on the Falkland Islands — wandering albatross, blue petrel, northern giant petrel, white-chinned petrel and grey-headed albatross (5, 7, 10, 19, 27 in Table 6 – 1; see sampling range in Supplementary Information SI. 6 – 1; scientific names in Table 6 – 1) — only one species — northern giant petrel showed significant F_{st}. Moreover, the F_{st} observed in the northern giant petrel was lower than that in the southern giant petrel which was sampled on the Falkland Islands (0.207 vs. 0.572).

Genetic differentiation was also observed within five Southern Temperate species occupying regions that were strongly influenced by Pleistocene climate fluctuations. Four of these species were located around Tasmania and New Zealand—regions that experienced glaciation and dramatic changes to coastline configuration during the Pleistocene – flesh-footed shearwater, Cook's petrel, yellow-eyed penguin and short-tailed shearwater, (1, 21, 43 in Figure 6 – 1; 3 short-tailed shearwater in Table 6 – 1; scientific names in Table 6 – 1). Glaciers periodically divided eastern and western regions of New Zealand during the Pleistocene, and likewise Cook's Strait was periodically closed (Proctor and Carter, 1989; Williams *et al*., 2009). These processes have been invoked for the isolation of invertebrate and plant lineages (Leschen *et al*., 2008; Wallis and Trewick, 2009; McCulloch *et al*., 2010; Fraser *et al*., 2012). Similarly, the exposure of Bassian Isthmus between Tasmania and mainland Australia during Pleistocene glaciations may have contributed to the isolation of eastern and western Australian seabird lineages, which were also potentially displaced northwards (Burridge, 2000; Fraser *et al*., 2009, 2012).

Variation in migratory status and non-breeding distributions do not predict genetic differentiation

Seabird colonies that migrate to population-specific non-breeding areas or remain resident near colonies year-round may have less opportunity for gene flow than those that have a shared non-breeding distributions or simply disperse widely during the non-breeding season (Friesen *et al*., 2007; Friesen, 2015). My GLMs failed to observe any such relationship. While some species with distinct non-breeding distributions exhibited high genetic structuring, this appeared to reflect other historic factors. For example, after removal of species lacking mutation-drift equilibrium, all of Southern Temperate species with distinct non-breeding distributions and significant genetic differentiation either breed in refuge zones or were located in New Zealand. In the case of the white-chinned petrel, individuals breeding on Crozet Island have been observed to winter on the coast of South Africa and individuals breeding in South Georgia use the northern Patagonian Shelf (Weimerskirch *et al*., 1999), yet these populations are genetically similar, whereas populations elsewhere in the species' range differ genetically. Therefore, historic factors may better explain seabird genetic structuring, as described above. These results underline our previous conclusion that distinguishing historical and biotic processes is crucial to predicting genetic divergence among seabird colonies.

Correlation between the number of sampling sites and genetic structure

I observed a significant positive relationship between F_{st} and the number of sample sites in Southern Temperate species. Studies sampling more seabird colonies are more likely to find signatures of genetic differentiation. However, the number of individuals and the distance among colonies sampled never appeared significant in any GLMs in this study, as might be expected if publication submission bias was operating. Publication submission bias (Møller

and Jennions, 2001), which reflects, *inter alia*, that authors may be more likely to submit studies refuting null hypotheses than studies that did not (Coursol and Wagner, 1986), could also be a factor. Publication submission bias is difficult to test directly as it requires information on unpublished datasets. Similarly, a lack of genetic differentiation is still of interest from evolutionary and conservation perspectives, and differentiation can be tested with small samples (e.g., two colonies).

Most biotic factors do not predict genetic structuring among seabird colonies

Except for the number of sample sites in Southern Temperate species, my GLMs failed to detect any relationship between population genetic differentiation and most factors such as marine distance among colonies, geographic region and population size, in addition to variation in migratory status and non-breeding distributions discussed earlier. This may reflect recent findings based on multispecies studies (>2,8 million locations from >2,600 tracked individuals across 50 marine vertebrates) suggesting that differences in movement patterns of large marine vertebrates are primarily defined by the species-specific traits and habitat, resulting in a large variability in movement patterns among individuals of the same species (Sequeira *et al*., 2018). The complexity of these internal factors, which are independent of the phylogenetic history or traits shared at higher taxonomic or functional groupings (i.e. family or taxa; Sequeira *et al*., 2018), are likely to explain the lack of relationship between most of the predictors tested in this study and genetic differentiation among seabird colonies, including the taxonomic family. One example is the apparent large-scale overlap in non-breeding distributions among colonies of Cory's shearwater breeding in Berlengas and in the Selvagens, two colonies in contrasting environments, exhibiting differences in timing, route and staging areas during migration (Catry *et al*., 2011). Moreover, as the extent of anthropogenic impacts on seabird species partly depends on the

animals' movement pattern (Sequeira *et al*., 2018), it is not surprising that our models also failed to detect any relationship between threat status and population genetic differentiation. This indicates that IUCN criteria do not encapsulate the elevated conservation priority for seabird species with genetically structured populations.

Conservation implications for seabirds

Seabirds are more threatened than any other group of birds and their status has deteriorated rapidly over recent decades mainly due to invasive species, bycatch, pollution and overfishing (Croxall *et al*., 2012). To cope with these pressures and adapt to environmental changes, seabird species need to maintain high levels of genetic diversity, which is best achieved by dispersal among colonies (Frankham, 1996), and hence, my study aimed to identify predictors of population genetic differentiation. However, for a high proportion of species, mtDNA variation is not at mutation-drift equilibrium, indicating likely historical influences on population genetic differentiation. Therefore, patterns of genetic variation may not always be reflective of contemporary processes, and mtDNA studies should consider the potential influence of historical factors to avoid overestimating contemporary connectivity (Avise & Hamrick, 1996) or falsely inferring factors that influence it.

Future directions

Results of the present study highlight several research needs. (i) Although my results show that most biotic factors do not predict genetic structuring among seabird colonies, proximity to the Falkland Islands appeared as a potential contributor to the development of genetic divergence in Southern Temperate seabird species. Hence, further studies should test the general importance of proximity to fertile foraging zones as a predictor of genetic structure among seabird colonies. (ii) Correlations between genetic structure, historical and abiotic

processes and phenotypic variation observed in seabirds needs to be investigated using other type of organisms as they can inform the origin and maintenance of genetic barriers among populations and species. (iii) Finally, as mtDNA can be under strong selection and evolve under unusual evolutionary rules (Ballard & Whitlock, 2004), similar GLMs should be conducted on nuclear datasets. As nuclear genes can be selected from distinct chromosomes, they can encapsulate stochastic variation of gene histories (Moore, 1995). Consequently, using multiple genetic markers, or loci, is often advocated, even for intra-specific studies, to provide an accurate perspective on an organism's evolutionary history (Ballard and Whitlock, 2004). However, presently these datasets greatly lag behind mtDNA studies in abundance.

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Supplementary Information SI. 6

Historical and physical factors dominate biotic processes as determinants of seabird population genetic differentiation

Anicee J. Lombal[1,4], James E. O'Dwyer[1,5], Vicki Friesen[2,6], Eric Woehler[2,7] and Christopher P. Burridge[1,8].

[1]School of Biological Sciences, University of Tasmania, Hobart, TAS 7001, Australia. Phone: +61 3 6226 7653.

[2]Department of Biology, Queen's University, Kingston, OL K7L 3N6, Canada. Phone:+61 3 533 6617

[3]Institute for Marine and Antarctic Studies, University of Tasmania, Hobart, TAS 7053, Australia. Phone: +61 3 6226 6379

[4]Anicee.Lombal@utas.edu.au

[5]18088076@students.latrobe.edu.au

[6]Vlf@queensu.ca

[7]Eric.Woehler@utas.edu.au

[8]Chris.Burridge@utas.edu.au

The following sections are included in this Supplementary Information SI. 6:

SI. 6 – 1 Location of sample sites for 73 seabird species as obtained in the literature. Genetic clusters are reported for each species as obtained in the seabird genetic studies. The two most distant sample sites are reported as inferred through the National Hurricane Centre (http://www.nhc.noaa.gov/gccalc.shtml).

Study n °	Publication	Species	Scientific name	Sampling sites	Latitude	Longitude	Oceanographic Region	Genetic cluster	Most distant sample sites
1	Lombal *et al*., 2018	flesh-footed shearwater	*Ardenna carneipes*	Ned's Beach, Lord Howe Island, Australia	-31.51	159.07	South Pacific Ocean	A	1
1	Lombal *et al*., 2018	flesh-footed shearwater	*Ardenna carneipes*	Clear Place, Lord Howe Island, Australia	-31.52	159.08	South Pacific Ocean	A	
1	Lombal *et al*., 2018	flesh-footed shearwater	*Ardenna carneipes*	Middle Beach, Lord Howe Island, Australia	-31.52	159.07	South Pacific Ocean	A	
1	Lombal *et al*., 2018	flesh-footed shearwater	*Ardenna carneipes*	Lady Alice Island, New Zealand	-35.54	174.44	South Pacific Ocean	A	
1	Lombal *et al*., 2018	flesh-footed shearwater	*Ardenna carneipes*	Coromandel Peninsula, New Zealand	-36.8	175.48	South Pacific Ocean	B	
1	Lombal *et al*., 2018	flesh-footed shearwater	*Ardenna carneipes*	Lewis Island, South Australia	-34.57	136.01	South Pacific Ocean	B	
1	Lombal *et al*., 2018	flesh-footed shearwater	*Ardenna carneipes*	Smith Island, South Australia	-35	136.01	South Pacific Ocean	C	
1	Lombal *et al*., 2018	flesh-footed shearwater	*Ardenna carneipes*	Shelter Island, Western Australia	-35.03	117.41	South Pacific Ocean	C	
1	Lombal *et al*., 2018	flesh-footed shearwater	*Ardenna carneipes*	Sandy Island, Western Australia	-34.51	116.02	South Pacific Ocean	C	
1	Lombal *et al*., 2018	flesh-footed shearwater	*Ardenna carneipes*	Breaksea Island, Western Australia	-35.04	118.03	South Pacific Ocean	C	
1	Lombal *et al*., 2018	flesh-footed shearwater	*Ardenna carneipes*	Coffin Island, Western Australia	-35	118.12	South Pacific Ocean	C	
1	Lombal *et al*., 2018	flesh-footed shearwater	*Ardenna carneipes*	Saint Paul Island	-38.84	77.83	Indian Ocean	C	2
2	Genovart *et al*., 2012	balearic shearwater	*Ardenna mauretanicus*	Menorca	39.95	4.1	Balearic Sea	A	1
2	Genovart *et al*., 2012	balearic shearwater	*Ardenna mauretanicus*	Malgrats, Mallorca	39.49	2.44	Balearic Sea	B	
2	Genovart *et al*., 2012	balearic shearwater	*Ardenna mauretanicus*	Sa Cella, Mallorca	39.59	2.65	Balearic Sea	B	
2	Genovart *et al*., 2012	balearic shearwater	*Ardenna mauretanicus*	Blanquer, Cabrera	39.18	2.96	Balearic Sea	B	
2	Genovart *et al*., 2012	balearic shearwater	*Ardenna mauretanicus*	Llumeta, Cabrera	39.16	2.97	Balearic Sea	B	
2	Genovart *et al*., 2012	balearic shearwater	*Ardenna mauretanicus*	Es Corral, Cabrera	39.13	2.93	Balearic Sea	B	
2	Genovart *et al*., 2012	balearic shearwater	*Ardenna mauretanicus*	Ila Conillera, Pitiüses	39.02	1.29	Balearic Sea	B	2
2	Genovart *et al*., 2012	balearic shearwater	*Ardenna mauretanicus*	Es Bosc, Pitiüses	38.99	1.19	Balearic Sea	B	
2	Genovart *et al*., 2012	balearic shearwater	*Ardenna mauretanicus*	Espardell, Pitiüses	38.78	1.42	Balearic Sea	B	
2	Genovart *et al*., 2012	balearic shearwater	*Ardenna mauretanicus*	Dragonera, Mallorca	39.58	2.31	Balearic Sea	B	
3	Austin *et al*., 1994	short-tailed shearwater	*Ardenna tenuirostris*	Whalebone Point, Australia	-43.28	147.14	Bass Strait	A	
3	Austin *et al*., 1994	short-tailed shearwater	*Ardenna tenuirostris*	Cape Deslacs, Australia	-42.98	147.54	Bass Strait	A	
3	Austin *et al*., 1994	short-tailed shearwater	*Ardenna tenuirostris*	Cape Direction, Australia	-43.06	147.25	Bass Strait	A	
3	Austin *et al*., 1994	short-tailed shearwater	*Ardenna tenuirostris*	Trial Harbour, Australia	-41.55	145.09	Bass Strait	A	1
3	Austin *et al*., 1994	short-tailed shearwater	*Ardenna tenuirostris*	Little Green Island, Australia	-40.13	148.15	Bass Strait	A	
3	Austin *et al*., 1994	short-tailed shearwater	*Ardenna tenuirostris*	Great Dog Island, Australia	-40.15	148.14	Bass Strait	A	
3	Austin *et al*., 1994	short-tailed shearwater	*Ardenna tenuirostris*	Port Fairy, Australia	-38.24	142.15	Bass Strait	A	

3	Austin *et al*., 1994	short-tailed shearwater	*Ardenna tenuirostris*	Cape Woolamai, Australia	-38.34	145.22	Bass Strait	A	
3	Austin *et al*., 1994	short-tailed shearwater	*Ardenna tenuirostris*	Doughboy Island, Australia	-38.46	146.17	Bass Strait	A	
3	Austin *et al*., 1994	short-tailed shearwater	*Ardenna tenuirostris*	Gabo Island, Australia	-37.34	149.55	Bass Strait	A	
3	Austin *et al*., 1994	short-tailed shearwater	*Ardenna tenuirostris*	Mantague Island, Australia	-36.15	150.13	Bass Strait	A	2
4	Gómez-Díaz *et al*., 2009	Cory's shearwater	*Calonectris diomedea*	Mallorca, Balearic Islands[δ]	39.58	2.36	Balearic Sea	A	
4	Gómez-Díaz*et al*., 2009	Cory's shearwater	*Calonectris diomedea*	Menorca, Balearic Islands[δ]	39.8	4.28	Balearic Sea	A	
4	Gómez-Díaz*et al*., 2009	Cory's shearwater	*Calonectris diomedea*	Ibiza, Balearic Islands[δ]	38.96	1.19	Balearic Sea	A	
4	Gómez-Díaz*et al*., 2009	Cory's shearwater	*Calonectris diomedea*	Cabrera, Balearic Islands[δ]	39.2	2.97	Balearic Sea	A	
4	Gómez-Díaz*et al*., 2009	Cory's shearwater	*Calonectris diomedea*	Columbretes, Spain[δ]	39.85	0.65	Mediterranean	A	
4	Gómez-Díaz*et al*., 2009	Cory's shearwater	*Calonectris diomedea*	Murcia, Spain[δ]	37.58	-0.98	Mediterranean	A	
4	Gómez-Díaz*et al*., 2009	Cory's shearwater	*Calonectris diomedea*	Tremiti, Italy[δ]	42.12	15.49	Mediterranean	A	
4	Gómez-Díaz*et al*., 2009	Cory's shearwater	*Calonectris diomedea*	Tuscany, Italy[δ]	42.4	11.86	Mediterranean	A	
4	Gómez-Díaz*et al*., 2009	Cory's shearwater	*Calonectris diomedea*	Sardinia, Italy[δ]	41.07	8.26	Mediterranean	A	
4	Gómez-Díaz*et al*., 2009	Cory's shearwater	*Calonectris diomedea*	Linosa, Italy[δ]	35.86	12.86	Mediterranean	A	
4	Gómez-Díaz*et al*., 2009	Cory's shearwater	*Calonectris diomedea*	Hyeres, France[δ]	43	6.21	Mediterranean	A	
4	Gómez-Díaz*et al*., 2009	Cory's shearwater	*Calonectris diomedea*	Chafarinas, Morocco[δ]	35.18	-2.41	Mediterranean	A	
4	Gómez-Díaz*et al*., 2009	Cory's shearwater	*Calonectris diomedea*	Crete, Greece[δ]	36.44	25.22	Mediterranean	A	1
4	Gómez-Díaz*et al*., 2009	Cory's shearwater	*Calonectris diomedea*	St. Maria, Azores Islands[δ]	36.94	-25.17	North Atlantic Ocean	B	
4	Gómez-Díaz*et al*., 2009	Cory's shearwater	*Calonectris diomedea*	Graciosa, Azores Islands[δ]	39.05	-27.95	North Atlantic Ocean	B	
4	Gómez-Díaz*et al*., 2009	Cory's shearwater	*Calonectris diomedea*	Corvo, Azores Islands[δ]	39.67	-31.1	North Atlantic Ocean	B	
4	Gómez-Díaz*et al*., 2009	Cory's shearwater	*Calonectris diomedea*	Faial, Azores Islands[δ]	38.52	-28.74	North Atlantic Ocean	B	
4	Gómez-Díaz*et al*., 2009	Cory's shearwater	*Calonectris diomedea*	Flores, Azores Islands[δ]	39.37	-31.19	North Atlantic Ocean	B	
4	Gómez-Díaz*et al*., 2009	Cory's shearwater	*Calonectris diomedea*	Sao Miguel, Azores Islands[δ]	37.7	-25.44	North Atlantic Ocean	B	
4	Gómez-Díaz*et al*., 2009	Cory's shearwater	*Calonectris diomedea*	Madeira, Portugal[δ]	32.34	-16.48	North Atlantic Ocean	B	
4	Gómez-Díaz*et al*., 2009	Cory's shearwater	*Calonectris diomedea*	Selvagens, Portugal[δ]	30.13	-15.86	North Atlantic Ocean	B	
4	Gómez-Díaz*et al*., 2009	Cory's shearwater	*Calonectris diomedea*	Berlengas, Portugal[δ]	39.4	-9.49	North Atlantic Ocean	B	
4	Gómez-Díaz*et al*., 2009	Cory's shearwater	*Calonectris diomedea*	Gran Canaria, Canary Islands[δ]	27.84	-15.78	North Atlantic Ocean	B	
4	Gómez-Díaz*et al*., 2009	Cory's shearwater	*Calonectris diomedea*	Lanzarote, Canary Islands[δ]	29.29	-13.53	North Atlantic Ocean	B	
4	Gómez-Díaz*et al*., 2009	Cory's shearwater	*Calonectris diomedea*	Tenerife, Canary Islands[δ]	28.44	-16.23	North Atlantic Ocean	B	
4	Gómez-Díaz*et al*., 2009	Cory's shearwater	*Calonectris diomedea*	La Palma, Canary Island[δ]	28.78	-17.79	North Atlantic Ocean	B	2
4	Gómez-Díaz*et al*., 2009	Cory's shearwater	*Calonectris diomedea*	Almeria, Spain[δ]	37.34	-1.65	North Atlantic Ocean	B	
5	Burg & Croxall, 2004	wandering albatross	*Diomedea exulans*	Antipodes Island	-49.58	178.69	South Pacific Ocean	A	1
5	Burg & Croxall, 2004	wandering albatross	*Diomedea exulans*	Campbell Island	-52.29	169.05	South Pacific Ocean	A	
5	Burg & Croxall, 2004	wandering albatross	*Diomedea exulans*	Adams Island	-50.75	165.96	South Pacific Ocean	A	
5	Burg & Croxall, 2004	wandering albatross	*Diomedea exulans*	Crozet Island	-45.74	51.23	Indian Ocean	B	
5	Burg & Croxall, 2004	wandering albatross	*Diomedea exulans*	Marion Island	-46.59	37.56	South Atlantic Ocean	B	
5	Burg & Croxall, 2004	wandering albatross	*Diomedea exulans*	South Georgia	-53.55	-36.72	South Atlantic Ocean	B	
5	Burg & Croxall, 2004	wandering albatross	*Diomedea exulans*	Tristan da Cunha	-36.05	-12.63	South Atlantic Ocean	C	2
6	Kerr & Dove, 2013	northern fulmar	*Fulmarus glacialis*	Kenai Peninsula, US, Alaska[δ]	60	-150.239	North Pacific Ocean	A	

6	Kerr & Dove, 2013	northern fulmar	*Fulmarus glacialis*	Gulf of Alaska, US, Alaska[δ,]	57.763	-149.408	North Pacific Ocean	A		
6	Kerr & Dove, 2013	northern fulmar	*Fulmarus glacialis*	Chowiet Island, US, Alaska[δ,]	56.214	-156.691	North Pacific Ocean	A		
6	Kerr & Dove, 2013	northern fulmar	*Fulmarus glacialis*	Chagulak Island, US, Alaska[δ,]	55.363	-170.444	North Pacific Ocean	A		
6	Kerr & Dove, 2013	northern fulmar	*Fulmarus glacialis*	Adak Island, US, Alaska[δ,]	51.779	-176.621	North Pacific Ocean	A		
6	Kerr & Dove, 2013	northern fulmar	*Fulmarus glacialis*	Amchitka Island, US, Alaska[δ,]	51.817	-179.151	North Pacific Ocean	A	1	
6	Kerr & Dove, 2013	northern fulmar	*Fulmarus glacialis*	Saint Georges Island, US, Alaska[δ,]	53.242	-170.444	North Pacific Ocean	A		
6	Kerr & Dove, 2013	northern fulmar	*Fulmarus glacialis*	Chukchi Sea[δ,]	70.375	-171.808	North Pacific Ocean	A		
6	Kerr & Dove, 2013	northern fulmar	*Fulmarus glacialis*	Prince Leopold Island, Canada[δ,]	76.706	-89.017	North Atlantic Ocean	B		
6	Kerr & Dove, 2013	northern fulmar	*Fulmarus glacialis*	Devon Island, Canada[δ,]	75.719	-81.82	North Atlantic Ocean	B		
6	Kerr & Dove, 2013	northern fulmar	*Fulmarus glacialis*	Baffin Island, Canada[δ,]	66.438	-70.929	North Atlantic Ocean	B		
6	Kerr & Dove, 2013	northern fulmar	*Fulmarus glacialis*	Faxafloi, Iceland[δ,]	64.484	-22.989	North Atlantic Ocean	B		
6	Kerr & Dove, 2013	northern fulmar	*Fulmarus glacialis*	Faeroe Island, Denmark[δ,]	64.197	-6.797	North Atlantic Ocean	B		
6	Kerr & Dove, 2013	northern fulmar	*Fulmarus glacialis*	Bear Island, Norway[δ,]	74.458	19.116	North Atlantic Ocean	B		
6	Kerr & Dove, 2013	northern fulmar	*Fulmarus glacialis*	Nordkapp, Norway[δ,]	71.025	25.637	North Atlantic Ocean	B		
6	Kerr & Dove, 2013	northern fulmar	*Fulmarus glacialis*	Traenabanken, Norway[δ,]	67.566	12.359	North Atlantic Ocean	B	2	
7	Quillfeldt *et al*., 2017	blue petrel	*Halobaena caeruela*	Kerguelen Islands	-49.28	69.57	Indian Ocean	A	1	
7	Quillfeldt *et al*., 2017	blue petrel	*Halobaena caeruela*	South Georgia	-54	-38.02	South Atlantic Ocean	A	2	
8	Cagnon *et al*., 2004	European storm petrel	*Hydrobates pelagicus*	Faroe Islands	62.34	-7.14	North Atlantic Ocean	A	1	
8	Cagnon *et al*., 2004	European storm petrel	*Hydrobates pelagicus*	Banneg, France	48.2	-4.56	North Atlantic Ocean	A		
8	Cagnon *et al*., 2004	European storm petrel	*Hydrobates pelagicus*	Biarritz, France	43.66	-1.73	North Atlantic Ocean	A		
8	Cagnon *et al*., 2004	European storm petrel	*Hydrobates pelagicus*	Marseille, France	43.29	5.31	Mediterranean	B		
8	Cagnon *et al*., 2004	European storm petrel	*Hydrobates pelagicus*	Corsica, France	42.15	8.56	Mediterranean	B	2	
9	Techow *et al*., 2010	southern giant petrel	*Macronectes giganteus*	Isla Arce, Argentina	-44.9	-65.54	South Atlantic Ocean	B	1	
9	Techow *et al*., 2010	southern giant petrel	*Macronectes giganteus*	Isla Noir, Chile	-54.31	-73.28	South Atlantic Ocean	B		
9	Techow *et al*., 2010	southern giant petrel	*Macronectes giganteus*	Isla Gran Robredo, Argentina	-45	-66.14	South Atlantic Ocean	B		
9	Techow *et al*., 2010	southern giant petrel	*Macronectes giganteus*	Isla de Los Estados, Argentina	-54.51	-64.36	South Atlantic Ocean	B		
9	Techow *et al*., 2010	southern giant petrel	*Macronectes giganteus*	South Georgia	-54.05	-36.63	South Atlantic Ocean	A		
9	Techow *et al*., 2010	southern giant petrel	*Macronectes giganteus*	Low hump, Gough Island	-40.3	-9.92	South Atlantic Ocean	B		
9	Techow *et al*., 2010	southern giant petrel	*Macronectes giganteus*	Georges Island, Falkland Islands	-52.28	-59.75	South Atlantic Ocean	B		
9	Techow *et al*., 2010	southern giant petrel	*Macronectes giganteus*	King Georges, South Shetland	-61.89	-58.09	South Atlantic Ocean	B		
9	Techow *et al*., 2010	southern giant petrel	*Macronectes giganteus*	Heard Island	-52.86	73.33	Indian Ocean	B		
9	Techow *et al*., 2010	southern giant petrel	*Macronectes giganteus*	Crozet Island	-45.74	51.23	Indian Ocean	B		
9	Techow *et al*., 2010	southern giant petrel	*Macronectes giganteus*	Prince Edward Island	-46.64	37.91	Indian Ocean	B		
9	Techow *et al*., 2010	southern giant petrel	*Macronectes giganteus*	Macquarie Island	-54.11	158.51	South Pacific Ocean	C	2	
10	Techow *et al*., 2010	northern giant petrel	*Macronectes halli*	South Georgia	-54.05	-36.63	South Atlantic Ocean	A	1	
10	Techow *et al*., 2010	northern giant petrel	*Macronectes halli*	Crozet Island	-45.74	51.23	Indian Ocean	C		
10	Techow *et al*., 2010	northern giant petrel	*Macronectes halli*	Prince Edward Island	-46.64	37.91	Indian Ocean	C		
10	Techow *et al*., 2010	northern giant petrel	*Macronectes halli*	Kerguelen Islands	-48.78	69.29	Indian Ocean	B		
10	Techow *et al*., 2010	northern giant petrel	*Macronectes halli*	Macquarie Island	-54.11	158.51	South Pacific Ocean	C		

10	Techow *et al*., 2010	northern giant petrel	*Macronectes halli*	Chatham Island	-43.84	-176.71	South Pacific Ocean	B	
10	Techow *et al*., 2010	northern giant petrel	*Macronectes halli*	Campbell Island	-52.29	169.05	South pacific Ocean	C	
10	Techow *et al*., 2010	northern giant petrel	*Macronectes halli*	Auckland Island	-50.67	166.07	South pacific Ocean	C	
10	Techow *et al*., 2010	northern giant petrel	*Macronectes halli*	Antipodes Island	-49.58	178.69	South pacific Ocean	C	2
11	Smith & Friesen, 2007	band-rumped storm-petrel	*Oceanodroma castro*	Azores, Portugal (Hot season breeders[δ,])	37.87	-26.02	North Atlantic Ocean	A	1
11	Smith & Friesen, 2007	band-rumped storm petrel	*Oceanodroma castro*	Azores, Portugal (Cool season breeders)	37.87	-26.02	North Atlantic Ocean	B	
11	Smith & Friesen, 2007	band-rumped storm-petrel	*Oceanodroma castro*	Farilhoes, Portugal	39.51	-9.55	North Atlantic Ocean	C	
11	Smith & Friesen, 2007	band-rumped storm-petrel	*Oceanodroma castro*	Madeira, Portugal (Hot season breeders)	32.82	-17.09	North Atlantic Ocean	D	
11	Smith & Friesen, 2007	band-rumped storm petrel	*Oceanodroma castro*	Madeira, Portugal (Cool season breeders)	32.82	-17.19	North Atlantic Ocean	C	
11	Smith & Friesen, 2007	band-rumped storm-petrel	*Oceanodroma castro*	Cape Verde (Hot season breeders)[δ,]	15.37	-23.95	North Atlantic Ocean	E	
11	Smith & Friesen, 2007	band-rumped storm petrel	*Oceanodroma castro*	Cape Verde (Cool season breeders)[δ,]	15.37	-23.95	North Atlantic Ocean	E	
11	Smith & Friesen, 2007	band-rumped storm-petrel	*Oceanodroma castro*	Ascension Island	-7.42	-14.53	South Atlantic Ocean	Not included in analyses (n=3)	
11	Smith & Friesen, 2007	band-rumped storm-petrel	*Oceanodroma castro*	Galápagos Island (Hot season breeders)	0.49	-91.15	Eastern Pacific	F	
11	Smith & Friesen, 2007	band-rumped storm-petrel	*Oceanodroma castro*	Galápagos Island (Cool season breeders)	0.49	-91.15	Eastern Pacific	G	
11	Smith & Friesen, 2007	band-rumped storm-petrel	*Oceanodroma castro*	Hawaii	20.36	-155.66	North Pacific Ocean	H	
11	Smith & Friesen, 2007	band-rumped storm-petrel	*Oceanodroma castro*	Japan	35.88	138.54	North Pacific Ocean	I	2
12	Bicknell *et al*., 2012	Leach's storm petrel[Ψ]	*Oceanodroma leucorhoa*	Buldir Island, US, Alaska	52.21	175.55	North Pacific Ocean	A	1
12	Bicknell *et al*., 2012	Leach's storm petrel[Ψ]	*Oceanodroma leucorhoa*	St Lazaria Island, Newfoundland, US	56.59	-135.43	North Pacific Ocean	A	
12	Bicknell *et al*., 2012	Leach's storm petrel[Ψ]	*Oceanodroma leucorhoa*	Baccalieu Island, Newfoundland, US	48.07	-52.48	North Atlantic Ocean	B	
12	Bicknell *et al*., 2012	Leach's storm petrel[Ψ]	*Oceanodroma leucorhoa*	Gull Island, Newfounland, US	47.15	-52.46	North Atlantic Ocean	B	
12	Bicknell *et al*., 2012	Leach's storm petrel[Ψ]	*Oceanodroma leucorhoa*	Bon portage Island, Nova Scotia	43.28	-65.25	North Atlantic Ocean	B	
12	Bicknell *et al*., 2012	Leach's storm petrel[Ψ]	*Oceanodroma leucorhoa*	Vestmannaeyjar Island, Iceland	63.25	-20.17	North Atlantic Ocean	B	
12	Bicknell *et al*., 2012	Leach's storm petrel[Ψ]	*Oceanodroma leucorhoa*	Rost, Norway	67.31	12.05	North Atlantic Ocean	B	
12	Bicknell *et al*., 2012	Leach's storm petrel[Ψ]	*Oceanodroma leucorhoa*	North Rona, Scotland	59.07	-5.49	North Atlantic Ocean	B	
12	Bicknell *et al*., 2012	Leach's storm petrel[Ψ]	*Oceanodroma leucorhoa*	St Kilda, Scotland	57.49	-8.35	North Atlantic Ocean	B	2
13	Quillfeldt *et al*., 2017	thin-billed prion[Ψ]	*Pachyptila belcheri*	Ile Mayes, Kerguelen Islands	-49.28	69.57	Indian Ocean	A	1
13	Quillfeldt *et al*., 2017	thin-billed prion[Ψ]	*Pachyptila belcheri*	Malvinas Islands, Falkland Islands	-51.43	-61.18	South Atlantic Ocean	A	2
14	Quillfeldt *et al*., 2017	Antarctic prion	*Pachyptila desolata*	Ile Verte, Kerguelen Islands	-49.3	70.02	Indian Ocean	A	1
14	Quillfeldt *et al*., 2017	Antarctic prion	*Pachyptila desolata*	South Georgia	-54	-38.02	South Atlantic Ocean	A	2
15	Ovenden *et al*., 1991	fairy prion	*Pachyptila turtur*	Flat Top Island, Australia	-43.38	146.23	South Pacific Ocean	A	
15	Ovenden *et al*., 1991	fairy prion	*Pachyptila turtur*	Tasman Island, Australia	-43.14	147.56	South Pacific Ocean	A	1
15	Ovenden *et al*., 1991	fairy prion	*Pachyptila turtur*	Albatross Island, Australia	-40.23	144.39	South Pacific Ocean	A	2
16	Silva *et al*., 2015	white-faced storm-petrel	*Pelagodroma marina*	Madeira, Portugal	32.84	-17.09	North Atlantic Ocean	A	1
16	Silva *et al*., 2015	white-faced storm-petrel	*Pelagodroma marina*	Mokohinau, New Zealand	-35.9	175.11	South Pacific Ocean	B	
16	Silva *et al*., 2015	white-faced storm-petrel	*Pelagodroma marina*	Gough Island	-39.71	-10.47	South Atlantic Ocean	C	2
17	Young, 2010	Laysan albatross	*Phoebastria immutabilis*	Wake Island, Hawaii	19.28	166.65	North Pacific Ocean	A	
17	Young, 2010	Laysan albatross	*Phoebastria immutabilis*	Kure Atoll, Hawaii	28.4	-178.29	North Pacific Ocean	A	1
17	Young, 2010	Laysan albatross	*Phoebastria immutabilis*	Midway Atoll, Hawaii	28.2	-177.36	North Pacific Ocean	B	
17	Young, 2010	Laysan albatross	*Phoebastria immutabilis*	Pearl & Hermes, Hawaii	27.83	-175.83	North Pacific Ocean	A	

17	Young, 2010	Laysan albatross	*Phoebastria immutabilis*	Laysan Island, Hawaii	25.88	-171.82	North Pacific Ocean	A	
17	Young, 2010	Laysan albatross	*Phoebastria immutabilis*	French Frigate Shoals, Hawaii	24.07	-166.28	North Pacific Ocean	A	
17	Young, 2010	Laysan albatross	*Phoebastria immutabilis*	Mukojima, Japan	35.8	139.78	North Pacific Ocean	A	
17	Young, 2010	Laysan albatross	*Phoebastria immutabilis*	Lehua, Hawaii	22.026	-160.11	North Pacific Ocean	A	
17	Young, 2010	Laysan albatross	*Phoebastria immutabilis*	Kauai, Hawaii	22.1	-159.52	North Pacific Ocean	A	
17	Young, 2010	Laysan albatross	*Phoebastria immutabilis*	Oahu, Hawaii	21.44	-158	North Pacific Ocean	A	
17	Young, 2010	Laysan albatross	*Phoebastria immutabilis*	Guadalupe, Mexico	29.24	-118.46	North Pacific Ocean	A	2
18	Walsh & Edwards, 2005	black-footed albatross	*Phoebastria nigripes*	French Frigate Shoals, Hawaii, US	23.45	-166.15	North Pacific Ocean	A	1
18	Walsh & Edwards, 2005	black-footed albatross	*Phoebastria nigripes*	Laysan Island, Hawaii, US	25.42	-171.44	North Pacific Ocean	A	
18	Walsh & Edwards, 2005	black-footed albatross	*Phoebastria nigripes*	Midway Atoll, Hawaii, US	28.12	-177.2	North Pacific Ocean	A	
18	Walsh & Edwards, 2005	black-footed albatross	*Phoebastria nigripes*	Izu Island, Torishima, Japan	30.29	-140.18	North Pacific Ocean	B	2
19	Techow *et al*., 2009	white-chinned petrel	*Procellaria aequinoctialis*	South Georgia	-54.05	-36.63	South Atlantic Ocean	A	1
19	Techow *et al*., 2009	white-chinned petrel	*Procellaria aequinoctialis*	Marion Island	-46.59	37.56	South Atlantic Ocean	A	
19	Techow *et al*., 2009	white-chinned petrel	*Procellaria aequinoctialis*	Crozet Island	-45.74	51.23	Indian Ocean	A	
19	Techow *et al*., 2009	white-chinned petrel	*Procellaria aequinoctialis*	Antipodes Island	-49.58	178.69	South Pacific Ocean	B	2
19	Techow *et al*., 2009	white-chinned petrel	*Procellaria aequinoctialis*	Auckland Island	-50.67	166.07	South Pacific Ocean	B	
20	Morris-Pocock *et al*., 2011	Trindade petrel	*Pterodroma arminjoniana*	Round Island	-19.56	57.7	Indian Ocean	A	1
20	Morris-Pocock *et al*., 2011	Trindade petrel	*Pterodroma arminjoniana*	Trindade Island	-19.43	-29.49	South Atlantic Ocean	B	2
21	Rayner *et al*., 2010	Cook's petrel	*Pterodroma cookii*	Codfish Island, New Zealand	-46.75	167.63	South Pacific Ocean	A	1
21	Rayner *et al*., 2010	Cook's petrel	*Pterodroma cookii*	Little Barrier Island, New Zealand	-36.15	175.05	South Pacific Ocean	B	2
22	Gangloff *et al*., 2013	gadfly petrel	*Pterodroma mollis*	Madeira, Portugal[δ]	32.84	-17.09	North Atlantic Ocean	A	1
22	Gangloff *et al*., 2013	gadfly petrel	*Pterodroma mollis*	Cape Verde[δ]	15.07	-23.58	North Atlantic Ocean	B	2
22	Gangloff *et al*., 2013	gadfly petrel	*Pterodroma mollis*	Bugio Island[δ]	32.44	-16.49	North Atlantic Ocean	C	
23	Welch *et al*., 2011	Galápagos petrel	*Pterodroma phaeopygia*	Floreana, Galápagos	-1.31	-90.43	Tropics	A	
23	Welch *et al*., 2011	Galápagos petrel	*Pterodroma phaeopygia*	Isabela, Galápagos	-0.66	-91.13	Tropics	B	1
23	Welch *et al*., 2011	Galápagos petrel	*Pterodroma phaeopygia*	San Cristobal, Galápagos	-0.87	-89.44	Tropics	C	2
23	Welch *et al*., 2011	Galápagos petrel	*Pterodroma phaeopygia*	Santa Cruz, Galápagos	-0.49	-90.28	Tropics	D	
23	Welch *et al*., 2011	Galápagos petrel	*Pterodroma phaeopygia*	Santiago, Galápagos	-0.24	-90.71	Tropics	B	
24	Wiley *et al*., 2012	Hawaiian petrel	*Pterodroma sandwichensis*	Hawaii	19.61	-155.55	North Pacific Ocean	A	1
24	Wiley *et al*., 2012	Hawaiian petrel	*Pterodroma sandwichensis*	Kauai	22.22	-159.53	North Pacific Ocean	B	2
25	Lombal *et al*., 2017	providence petrel	*Pterodroma solandri*	Far flat, Lord Howe Island, Australia	-31.56	159.07	South Pacific Ocean	A	1
25	Lombal *et al*., 2017	providence petrel	*Pterodroma solandri*	Mt Gower, Lord Howe Island, Australia	-31.58	159.07	South Pacific Ocean	A	
25	Lombal *et al*., 2017	providence petrel	*Pterodroma solandri*	George's Bay, Lord Howe Island, Australia	-31.56	159.09	South Pacific Ocean	A	
25	Lombal *et al*., 2017	providence petrel	*Pterodroma solandri*	Muttonbird Point, Lord Howe Island, Australia	-31.54	159.09	South Pacific Ocean	A	
25	Lombal *et al*., 2017	providence petrel	*Pterodroma solandri*	Phillip Island, Norfolk Island Group, Australia	-29.12	167.95	South Pacific Ocean	A	2
26	Abbott & Double, 2003b	shy albatross	*Thalassarche cauta*	Albatross Island, Australia	-40.34	144.66	Tasman Sea	A	1
26	Abbott & Double, 2003b	shy albatross	*Thalassarche cauta*	Mewstone, Australia	-43.69	146.32	Tasman Sea	A	
26	Abbott & Double, 2003b	shy albatross	*Thalassarche cauta*	Pedra Branca, Australia	-43.81	146.94	Tasman Sea	A	2

27	Burg & Croxall, 2001	grey-headed albatross	*Thalassarche chrysostoma*	Diego Ramirez Islands	-56.36	-68.72	Drake passage	A	1
27	Burg & Croxall, 2001	grey-headed albatross	*Thalassarche chrysostoma*	South Georgia	-54.05	-36.63	South Atlantic Ocean	A	
27	Burg & Croxall, 2001	grey-headed albatross	*Thalassarche chrysostoma*	Marion Island	-46.01	37.46	Indian Ocean	A	
27	Burg & Croxall, 2001	grey-headed albatross	*Thalassarche chrysostoma*	Kerguelen Islands	-48.78	69.29	Indian Ocean	A	
27	Burg & Croxall, 2001	grey-headed albatross	*Thalassarche chrysostoma*	Campbell Island	-52.13	169.05	South pacific Ocean	A	2
28	Burg & Croxall, 2001	black-browed albatross	*Thalassarche melanophris*	Diego Ramirez Islands	-56.36	-68.72	Drake passage	A	1
28	Burg & Croxall, 2001	black-browed albatross	*Thalassarche melanophris*	Falkland Islands	-51.77	-59.23	South Atlantic Ocean	B	
28	Burg & Croxall, 2001	black-browed albatross	*Thalassarche melanophris*	South Georgia	-54.05	-36.63	South Atlantic Ocean	A	
28	Burg & Croxall, 2001	black-browed albatross	*Thalassarche melanophris*	Kerguelen Islands	-48.78	69.29	Indian Ocean	A	
28	Burg & Croxall, 2001	black-browed albatross	*Thalassarche melanophris*	Campbell Island (sp1)	-52.13	169.05	South Pacific Ocean	A	2
28	Burg & Croxall, 2001	black-browed albatross	*Thalassarche melanophris*	Campbell Island (sp2)[δ]	-52.13	169.05	South Pacific Ocean	C	
29	Abbott & Double, 2003b	white-capped albatross	*Thalassarche steadi*	Disappointment Island, New Zealand	-50.27	165.97	Tasman Sea	A	1
29	Abbott & Double, 2003b	white-capped albatross	*Thalassarche steadi*	Southwest Cape, New Zealand	-50.83	165.88	Tasman Sea	A	
29	Abbott & Double, 2003b	white-capped albatross	*Thalassarche steadi*	Logan Point, New Zealand	-50.85	165.9	Tasman Sea	A	2
30	Steeves *et al*., 2003	masked booby	*Sula dactylatra*	Johnston atoll, US	17.23	-169.88	Central Pacific	A	1
30	Steeves *et al*., 2003	masked booby	*Sula dactylatra*	Isla San Benedicto, Mexico	20.3	-111.15	Eastern Pacific	A	
30	Steeves *et al*., 2003	masked booby	*Sula dactylatra*	Clipperton Atoll	10.68	-109.32	Eastern Pacific	A	
30	Steeves *et al*., 2003	masked booby	*Sula dactylatra*	Monito Island, Puerto Rico	18.53	-67.94	Caribbean	B	2
31	Levin & Parker, 2012	nazca booby	*Sula grandi*	Darwin, Galápagos	1.67	-92	Tropics	A	1
31	Levin & Parker, 2012	nazca booby	*Sula grandi*	Espagnola, Galápagos	-1.35	-89.68	Tropics	B	2
31	Levin & Parker, 2012	nazca booby	*Sula grandi*	Genovesa Island, Ecuador	0.2	-89.57	Tropics	B	
31	Levin & Parker, 2012	nazca booby	*Sula grandi*	San Cristobal, Galápagos	-0.82	-89.46	Tropics	C	
31	Levin & Parker, 2012	nazca booby	*Sula grandi*	Wolf Island, Ecuador	1.43	-91.82	Tropics	A	
32	Morris-Pocock *et al*., 2010	brown booby	*Sula leucogaster*	Monito Island, Puerto Rico	18.05	-67.53	Caribbean Sea	A	
32	Morris-Pocock *et al*., 2010	brown booby	*Sula leucogaster*	Cape Verde	15.05	-24.48	North Atlantic Ocean	A	
32	Morris-Pocock *et al*., 2010	brown booby	*Sula leucogaster*	Ascension Island	-7.56	-14.22	South Atlantic Ocean	A	
32	Morris-Pocock *et al*., 2010	brown booby	*Sula leucogaster*	Christmas Island	-10.29	105.71	Indian Ocean	B	1
32	Morris-Pocock *et al*., 2010	brown booby	*Sula leucogaster*	Palmyra atoll, US	5.33	-162.5	Central Pacific	C	2
32	Morris-Pocock *et al*., 2010	brown booby	*Sula leucogaster*	Johnston atoll, US	16.45	-169.31	Central Pacific	C	
32	Morris-Pocock *et al*., 2010	brown booby	*Sula leucogaster*	Gorgona Island, Colombia	2.58	-78.1	Eastern Pacific	D	
32	Morris-Pocock *et al*., 2010	brown booby	*Sula leucogaster*	Isla San-Benedicto, Mexico	19.19	-110.49	Eastern Pacific	D	
32	Morris-Pocock *et al*., 2010	brown booby	*Sula leucogaster*	Piedra Blanca, California, US	21.25	-106.28	Eastern Pacific	D	
32	Morris-Pocock *et al*., 2010	brown booby	*Sula leucogaster*	Farallon de San Ignacio, Mexico	25.26	-109.22	Gulf of Califomia	E	
32	Morris-Pocock *et al*., 2010	brown booby	*Sula leucogaster*	San Pedro Martyr, Mexico	28.24	-112.16	Gulf of Califomia	E	
33	Taylor *et al*., 2011	blue-footed booby	*Sula nebouxii*	Isla San Ildefonso, Mexico	27.18	-111.43	Gulf of Califomia	A	1
33	Taylor *et al*., 2011	blue-footed booby	*Sula nebouxii*	Farallon de San Ignacio, Mexico	28.2	-111.59	Gulf of Califomia	A	
33	Taylor *et al*., 2011	blue-footed booby	*Sula nebouxii*	El rancho, Mexico	27.94	-111.02	Gulf of Califomia	A	
33	Taylor *et al*., 2011	blue-footed booby	*Sula nebouxii*	La Plata, Ecuador	-1.24	-81.07	Eastern Pacific	A	
33	Taylor *et al*., 2011	blue-footed booby	*Sula nebouxii*	Lobos de Tierra, Peru	-6.43	-80.86	Eastern Pacific	A	2

33	Taylor *et al*., 2011	blue-footed booby	*Sula nebouxii*	Champion Island, Galápagos	-1.36	-89.68	Tropics	A	
33	Taylor *et al*., 2011	blue-footed booby	*Sula nebouxii*	Seymour Island, Galápagos	-0.34	-90.29	Tropics	A	
33	Taylor *et al*., 2011	blue-footed booby	*Sula nebouxii*	Espanola Island, Galápagos	-1.37	-89.67	Tropics	A	
34	Morris-Pocock *et al*., 2010	red-footed booby	*Sula sula*	Monito Island, Puerto Rico	18.05	-67.53	Caribbean sea	A	
34	Morris-Pocock *et al*., 2010	red-footed booby	*Sula sula*	Fernando de Norhona, Brazil	3.52	-32.24	Atlantic	A	
34	Morris-Pocock *et al*., 2010	red-footed booby	*Sula sula*	Ascension Island	-7.56	-14.22	South Atlantic Ocean	A	
34	Morris-Pocock *et al*., 2010	red-footed booby	*Sula sula*	Aldabra atoll, Seychelles	-9.24	46.22	Indian Ocean	A	1
34	Morris-Pocock *et al*., 2010	red-footed booby	*Sula sula*	North Keeling Island	-12.07	96.54	Indian Ocean	A	
34	Morris-Pocock *et al*., 2010	red-footed booby	*Sula sula*	Herald Cays	-16.56	149.13	Pacific	A	
34	Morris-Pocock *et al*., 2010	red-footed booby	*Sula sula*	Palmyra atoll, US	5.33	-162.5	Central Pacific	B	
34	Morris-Pocock *et al*., 2010	red-footed booby	*Sula sula*	Johnston atoll, US	16.45	-169.31	Central Pacific	B	
34	Morris-Pocock *et al*., 2010	red-footed booby	*Sula sula*	Tern Island, Hawaii, US	23.52	-166.17	Central Pacific	B	
34	Morris-Pocock *et al*., 2010	red-footed booby	*Sula sula*	Genovesa Island, Ecuador	0.2	-89.57	Eastern Pacific	B	2
35	Taylor *et al*., 2011	Peruvian booby	*Sula variegata*	Lobos de Tierra, Peru	-6.43	-80.86	Eastern Pacific	A	1
35	Taylor *et al*., 2011	Peruvian booby	*Sula variegata*	Lobos de Afuera , Peru	-11.64	-77.01	Eastern Pacific	A	
35	Taylor *et al*., 2011	Peruvian booby	*Sula variegata*	Isla Mazorca	-11.06	-77.78	Eastern Pacific	A	
35	Taylor *et al*., 2011	Peruvian booby	*Sula variegata*	Isla Chincha Norte	-13.39	-76.52	Eastern Pacific	A	
35	Taylor *et al*., 2011	Peruvian booby	*Sula variegata*	Isla Pajaros, Panama	-29.43	-71.7	Eastern Pacific	A	2
36	Barlow *et al*., 2011	European cormorant	*Phalacrocorax aristotelis*	Hornoya, Norway	70.23	31.08	North Atlantic Ocean	A	1
36	Barlow *et al*., 2011	European cormorant	*Phalacrocorax aristotelis*	Rost, Norway	67.26	11.54	North Atlantic Ocean	A	
36	Barlow *et al*., 2011	European cormorant	*Phalacrocorax aristotelis*	Kjoer, Norway	58.53	5.26	North Atlantic Ocean	A	
36	Barlow *et al*., 2011	European cormorant	*Phalacrocorax aristotelis*	Flatey, Iceland	65.22	-22.54	North Atlantic Ocean	A	
36	Barlow *et al*., 2011	European cormorant	*Phalacrocorax aristotelis*	Skuvoy, Faroe	61.46	-6.49	North Atlantic Ocean	A	
36	Barlow *et al*., 2011	European cormorant	*Phalacrocorax aristotelis*	Isle of May, Scotland	56.11	-2.33	North Atlantic Ocean	B	
36	Barlow *et al*., 2011	European cormorant	*Phalacrocorax aristotelis*	Lambay, Ireland	53.29	-6.01	North Atlantic Ocean	B	
36	Barlow *et al*., 2011	European cormorant	*Phalacrocorax aristotelis*	Ile de Beniguet, France	48.5	-3.01	North Atlantic Ocean	C	
36	Barlow *et al*., 2011	European cormorant	*Phalacrocorax aristotelis*	Vizcaya, Spain	43.26	-2.56	North Atlantic Ocean	C	
36	Barlow *et al*., 2011	European cormorant	*Phalacrocorax aristotelis*	Galicia, Spain	42.13	-8.54	North Atlantic Ocean	C	
36	Barlow *et al*., 2011	European cormorant	*Phalacrocorax aristotelis*	Corsica, France	42.22	8.32	Mediterranean	C	2
37	Calderón *et al*., 2014	imperial shag$^{\Psi}$	*Phalacrocorax atriceps*	Punta Leon, Chubut, Argentina	-43.03	-64.28	South Atlantic Ocean	A	
37	Calderón *et al*., 2014	imperial shag$^{\Psi}$	*Phalacrocorax atriceps*	Roca Malaspina, Chubut, Argentina	-45.18	-66.51	South Atlantic Ocean	A	1
37	Calderón *et al*., 2014	imperial shag$^{\Psi}$	*Phalacrocorax atriceps*	Isla Chata, Santa Cruz, Argentina	-47.93	-65.73	South Atlantic Ocean	A	
37	Calderón *et al*., 2014	imperial shag$^{\Psi}$	*Phalacrocorax atriceps*	Pico Quebrado, Santa Cruz, Argentina	-50.25	-68.63	South Atlantic Ocean	A	
37	Calderón *et al*., 2014	imperial shag$^{\Psi}$	*Phalacrocorax atriceps*	New Island, Malvinas, Falkland Islands	-54.86	-68.23	Fuegian region	B	
37	Calderón *et al*., 2014	imperial shag$^{\Psi}$	*Phalacrocorax atriceps*	Becasses, Tierra del Fuego, Chili	-51.71	-61.28	Fuegian region	B	
37	Calderón *et al*., 2014	imperial shag$^{\Psi}$	*Phalacrocorax atriceps*	Punta arenas, XII region, Chili	-52.47	-69.57	Fuegian region	B	
37	Calderón *et al*., 2014	imperial shag$^{\Psi}$	*Phalacrocorax atriceps*	Lake Yehuin, Tierra del Fuego, Chili	-54.41	-67.7	Fuegian region	C	
37	Calderón *et al*., 2014	imperial shag$^{\Psi}$	*Phalacrocorax atriceps*	Lake Vintter, Chubut, Argentina	-43.93	-71.6	South Pacific Ocean	D	
37	Calderón *et al*., 2014	imperial shag$^{\Psi}$	*Phalacrocorax atriceps*	Llanquihue, X region, Chili	-41.8	-76.66	South Pacific Ocean	D	2

37	Calderón *et al*., 2014	imperial shag[Ψ]	*Phalacrocorax atriceps*	Lake N. Huapi, Rio negro, Chili	-40.98	-71.5	South Pacific Ocean	D		
38	Mercer *et al*., 2013	double-crested cormorant	*Phalacrocorax auritus*	Eastern Aleutians, Alaska, US	54.13	-165.82	North Pacific Ocean	A	1	
38	Mercer *et al*., 2013	double-crested cormorant	*Phalacrocorax auritus*	Kenai Peninsula, Alaska, US	61.24	-151.52	North Pacific Ocean	A		
38	Mercer *et al*., 2013	double-crested cormorant	*Phalacrocorax auritus*	Gray's Harbor, WA, US	47.72	-123.71	North Pacific Ocean	B		
38	Mercer *et al*., 2013	double-crested cormorant	*Phalacrocorax auritus*	Walla Walla, WA, US	46.07	-118.34	North Pacific Ocean	B		
38	Mercer *et al*., 2013	double-crested cormorant	*Phalacrocorax auritus*	Clatsop Co., OR, US	46.15	-124.02	North Pacific Ocean	B		
38	Mercer *et al*., 2013	double-crested cormorant	*Phalacrocorax auritus*	San-Francisco, CA, US	37.67	-122.59	North Pacific Ocean	B		
38	Mercer *et al*., 2013	double-crested cormorant	*Phalacrocorax auritus*	San Diego, CA, US	34.23	-117.26	North Pacific Ocean	B		
38	Mercer *et al*., 2013	double-crested cormorant	*Phalacrocorax auritus*	Imperial Co, CA, US	33.13	-115.73	Interior land	B		
38	Mercer *et al*., 2013	double-crested cormorant	*Phalacrocorax auritus*	Modoc Co, CA, US	41.92	-120.43	Interior land (lake)	B		
38	Mercer *et al*., 2013	double-crested cormorant	*Phalacrocorax auritus*	Caribou Co., ID, US	41.38	-112.82	Interior land (lake)	B		
38	Mercer *et al*., 2013	double-crested cormorant	*Phalacrocorax auritus*	Lakeland, AB, US	55.34	-115.21	Interior land (lake)	C		
38	Mercer *et al*., 2013	double-crested cormorant	*Phalacrocorax auritus*	Regina, SK, US	50.19	-106.25	Interior land (lake)	C		
38	Mercer *et al*., 2013	double-crested cormorant	*Phalacrocorax auritus*	Cass Co, MN, US	46.94	-91.3	Interior land (lake)	C		
38	Mercer *et al*., 2013	double-crested cormorant	*Phalacrocorax auritus*	Door Co., WI, US	44.98	-84.18	Interior land (lake)	C		
38	Mercer *et al*., 2013	double-crested cormorant	*Phalacrocorax auritus*	Albitibi, QC, US	49.11	-78.25	Interior land (lake)	C		
38	Mercer *et al*., 2013	double-crested cormorant	*Phalacrocorax auritus*	Buffalo Co., PA, US	43.32	-78.44	Interior land (lake)	C		
38	Mercer *et al*., 2013	double-crested cormorant	*Phalacrocorax auritus*	Pictou Co, NS, US	44.52	-63.63	North Atlantic Ocean	C		
38	Mercer *et al*., 2013	double-crested cormorant	*Phalacrocorax auritus*	Dukes Co., MA, US	41.52	-70.72	North Atlantic Ocean	C		
38	Mercer *et al*., 2013	double-crested cormorant	*Phalacrocorax auritus*	Talbot Co, MD, US	38.25	-75.25	North Atlantic Ocean	C		
38	Mercer *et al*., 2013	double-crested cormorant	*Phalacrocorax auritus*	Harrisburg Co., PA, US	40.19	-76.94	North Atlantic Ocean	C		
38	Mercer *et al*., 2013	double-crested cormorant	*Phalacrocorax auritus*	Sevier Co., AR, US	34.06	-94.33	Interior land	D		
38	Mercer *et al*., 2013	double-crested cormorant	*Phalacrocorax auritus*	Yazoo Co., MS, US	32.92	-90.42	Interior land	D		
38	Mercer *et al*., 2013	double-crested cormorant	*Phalacrocorax auritus*	Calhoun Co., SC, US	33.79	-80.7	Interior land	D	2	
39	Marion & Le Gentil, 2006	great cormorant	*Phalacrocorax carbo*	Lake of Grand Lieu, Brittany	47.24	-1.75	North Atlantic Ocean	A		
39	Marion & Le Gentil, 2006	great cormorant	*Phalacrocorax carbo*	Ile des Landes, Brittany	48.78	-1.83	North Atlantic Ocean	B		
39	Marion & Le Gentil, 2006	great cormorant	*Phalacrocorax carbo*	Briere, Brittany	47.36	-2.21	North Atlantic Ocean	A		
39	Marion & Le Gentil, 2006	great cormorant	*Phalacrocorax carbo*	Ile de Govihan, Brittany	47.62	-2.93	North Atlantic Ocean	B		
39	Marion & Le Gentil, 2006	great cormorant	*Phalacrocorax carbo*	Chausey Island, Normandy	48.95	-1.91	North Atlantic Ocean	B		
39	Marion & Le Gentil, 2006	great cormorant	*Phalacrocorax carbo*	Kwalnes, Lofoten, Norway	68.58	13.96	North Atlantic Ocean	D	1	
39	Marion & Le Gentil, 2006	great cormorant	*Phalacrocorax carbo*	Santa Caterina, Sardinia	40.75	14.57	Mediterranean	C	2	
39	Marion & Le Gentil, 2006	great cormorant	*Phalacrocorax carbo*	Oostvardersplassen, The Netherlands	52.4	5.41	North Atlantic Ocean	A		
39	Marion & Le Gentil, 2006	great cormorant	*Phalacrocorax carbo*	Svarto, Sweden	57.21	16.48	North Atlantic Ocean	A		
39	Marion & Le Gentil, 2006	great cormorant	*Phalacrocorax carbo*	Gomel, Belarus	53.21	30.28	North Atlantic Ocean	A		
39	Marion & Le Gentil, 2006	great cormorant	*Phalacrocorax carbo*	Punte Alberte, Italy	44.59	12.22	Mediterranean	A		
39	Marion & Le Gentil, 2006	great cormorant	*Phalacrocorax carbo*	Val Campotto, Italy	44.79	11.78	Mediterranean	A		
39	Marion & Le Gentil, 2006	great cormorant	*Phalacrocorax carbo*	Lerharim Island, Finland	53.36	-1.65	North Atlantic Ocean	A		
39	Marion & Le Gentil, 2006	great cormorant	*Phalacrocorax carbo*	Mageoerne, Denmark	55.95	9.07	North Atlantic Ocean	A		
39	Marion & Le Gentil, 2006	great cormorant	*Phalacrocorax carbo*	Yderste Holm, Denmark	56.68	9.95	North Atlantic Ocean	A		

39	Marion & Le Gentil, 2006	great cormorant	*Phalacrocorax carbo*	Ross-shire, Scotland	57.56	-4.99	North Atlantic Ocean	C		
39	Marion & Le Gentil, 2006	great cormorant	*Phalacrocorax carbo*	Kintyre, Scotland	55.81	-5.77	North Atlantic Ocean	C		
39	Marion & Le Gentil, 2006	great cormorant	*Phalacrocorax carbo*	Kiel, Germany	54.62	10.12	North Atlantic Ocean	C		
39	Marion & Le Gentil, 2006	great cormorant	*Phalacrocorax carbo*	St. Margaret's Island, Wales	51.69	-4.76	North Atlantic Ocean	C		
39	Marion & Le Gentil, 2006	great cormorant	*Phalacrocorax carbo*	Caithness, Scotland	58.23	-3.82	North Atlantic Ocean	C		
40	Calderón *et al*., 2014	rock shag	*Phalacrocorax magellanicus*	Punta Arco, Chubut, Argentina	-42.5	-64.5	South Atlantic Ocean	A		
40	Calderón *et al*., 2014	rock shag	*Phalacrocorax magellanicus*	Las Charas, Chubut, Argentina	-42.7	-64.98	South Atlantic Ocean	A		
40	Calderón *et al*., 2014	rock shag	*Phalacrocorax magellanicus*	Punta Loma, Chubut, Argentina	-42.81	-64.88	South Atlantic Ocean	A		
40	Calderón *et al*., 2014	rock shag	*Phalacrocorax magellanicus*	Roca Malaspina, Chubut, Argentina	-45.18	-66.51	South Atlantic Ocean	A		
40	Calderón *et al*., 2014	rock shag	*Phalacrocorax magellanicus*	Vernaci Este, Chubut, Argentina	-45.18	-66.48	South Atlantic Ocean	A		
40	Calderón *et al*., 2014	rock shag	*Phalacrocorax magellanicus*	Cabo Blanco, Santa Cruz, Argentina	-47.2	-65.75	Atlantic Coast	A		
40	Calderón *et al*., 2014	rock shag	*Phalacrocorax magellanicus*	Isla Elena, Santa Cruz, Argentina	-47.75	-65.93	Atlantic Coast	A		
40	Calderón *et al*., 2014	rock shag	*Phalacrocorax magellanicus*	Monte Leon, Santa Cruz, Argentina	-50.33	-68.88	Atlantic Coast	A	1	
40	Calderón *et al*., 2014	rock shag	*Phalacrocorax magellanicus*	Lively Island, Malvinas, Falkland Islands	-52.02	-58.46	Fuegian region	B		
40	Calderón *et al*., 2014	rock shag	*Phalacrocorax magellanicus*	Bahia Ushuaia, Tierra Del Fuego	-54.84	-68.25	Fuegian region	B		
40	Calderón *et al*., 2014	rock shag	*Phalacrocorax magellanicus*	Strait of Magellan, XII region, Chile	-52.47	-69.57	Fuegian region	B		
40	Calderón *et al*., 2014	rock shag	*Phalacrocorax magellanicus*	Llanquihue, X region, Chile	-41.8	-76.66	Pacific Coast	C	2	
41	Younger *et al*., 2015	emperor penguin	*Aptenodytes forsteri*	Halley Bay, Weddell Sea	-73.35	-26.39	Antarctic	A	1	
41	Younger *et al*., 2015	emperor penguin	*Aptenodytes forsteri*	Gould Bay, Weddell Sea	-77.43	-47.41	Antarctic	A		
41	Younger *et al*., 2015	emperor penguin	*Aptenodytes forsteri*	Fold Island, Prydz Bay	-72.48	69.86	Antarctic	A		
41	Younger *et al*., 2015	emperor penguin	*Aptenodytes forsteri*	Auster, Prydz Bay	-73.11	68.81	Antarctic	A		
41	Younger *et al*., 2015	emperor penguin	*Aptenodytes forsteri*	Amanda Bay, Prydz Bay	-77.49	76.76	Antarctic	A		
41	Younger *et al*., 2015	emperor penguin	*Aptenodytes forsteri*	Club Lake, Prydz Bay	-77.9	66.7	Antarctic	A	2	
41	Younger *et al*., 2015	emperor penguin	*Aptenodytes forsteri*	Pointe Geologie, Adélie Land	-66.66	139.55	Antarctic	A		
41	Younger *et al*., 2015	emperor penguin	*Aptenodytes forsteri*	Cape Crozier, Ross Sea	-77.27	169.13	Antarctic	B		
41	Younger *et al*., 2015	emperor penguin	*Aptenodytes forsteri*	Cape Washington, Ross Sea	-74.39	165.25	Antarctic	B		
42	Clucas *et al*., 2016	king penguin	*Aptenodytes patagonicus*	Volunteer point, Falkland Islands	-51.28	-57.5	South Atlantic Ocean	A	1	
42	Clucas *et al*., 2016	king penguin	*Aptenodytes patagonicus*	Fortuna Bay, South Georgia	-53.96	-36.73	South Atlantic Ocean	B		
42	Clucas *et al*., 2016	king penguin	*Aptenodytes patagonicus*	Baie du Marin, Possession Island, Crozet Island	-46.07	51.75	Indian Ocean	A		
42	Clucas *et al*., 2016	king penguin	*Aptenodytes patagonicus*	Sandy Bay, Macquarie Island	-53.96	158.68	South Pacific Ocean	A	2	
43	Boessenkool *et al*., 2009	yellow-eyed penguin[Ψ]	*Eudyptes antipodes*	Campbell Island	-52.32	169.05	South Pacific Ocean	A	1	
43	Boessenkool *et al*., 2009	yellow-eyed penguin[Ψ]	*Eudyptes antipodes*	Auckland Island	-50.29	166.17	South Pacific Ocean	A		
43	Boessenkool *et al*., 2009	yellow-eyed penguin[Ψ]	*Eudyptes antipodes*	North Otago, South New Zealand	-45.23	170.52	South Pacific Ocean	B	2	
43	Boessenkool *et al*., 2009	yellow-eyed penguin[Ψ]	*Eudyptes antipodes*	Otago peninsula, South New Zealand	-45.53	170.37	South Pacific Ocean	B		
43	Boessenkool *et al*., 2009	yellow-eyed penguin[Ψ]	*Eudyptes antipodes*	Catlins, South New Zealand	-46.34	169.35	South Pacific Ocean	B		
43	Boessenkool *et al*., 2009	yellow-eyed penguin[Ψ]	*Eudyptes antipodes*	Stewart Island, New Zealand	-46.57	168.8	South Pacific Ocean	B		
43	Boessenkool *et al*., 2009	yellow-eyed penguin[Ψ]	*Eudyptes antipodes*	Codfish Island, New Zealand	-46.46	167.38	South Pacific Ocean	B		
44	Banks *et al*., 2006	rockhopper penguin	*Eudyptes chrysocome*	Falkland Islands[δ]	-51.72	-61.31	Atlantic	A	1	
44	Banks *et al*., 2006	rockhopper penguin	*Eudyptes chrysocome*	Crozet Island	-45.89	51.41	Indian Ocean	B		

44	Banks *et al*., 2006	rockhopper penguin	*Eudyptes chrysocome*	Kerguelen Islands	-48.9	69.11	Indian Ocean	B	
44	Banks *et al*., 2006	rockhopper penguin	*Eudyptes chrysocome*	Amsterdam Island[δ,]	-37.66	77.46	Indian Ocean	C	2
45	Grosser *et al*., 2015	little penguin	*Eudyptula minor*	Auckland, New Zealand	-36.68	174.71	South Pacific Ocean	A	
45	Grosser *et al*., 2015	little penguin	*Eudyptula minor*	Bay of Plenty, New Zealand	-37.58	176.12	South Pacific Ocean	A	
45	Grosser *et al*., 2015	little penguin	*Eudyptula minor*	Hawke's Bay, New Zealand	-39.33	176.93	South Pacific Ocean	A	
45	Grosser *et al*., 2015	little penguin	*Eudyptula minor*	Wellington, New Zealand	-41.29	174.59	South Pacific Ocean	A	
45	Grosser *et al*., 2015	little penguin	*Eudyptula minor*	Golden Bay, New Zealand	-40.79	172.88	South Pacific Ocean	A	
45	Grosser *et al*., 2015	little penguin	*Eudyptula minor*	West Coast, New Zealand	-41.77	171.41	South Pacific Ocean	A	
45	Grosser *et al*., 2015	little penguin	*Eudyptula minor*	Kaikura, New Zealand	-42.41	173.68	South Pacific Ocean	A	
45	Grosser *et al*., 2015	little penguin	*Eudyptula minor*	Chatham Island, New Zealand	-43.9	-176.52	South Pacific Ocean	A	1
45	Grosser *et al*., 2015	little penguin	*Eudyptula minor*	Banks Peninsula, New Zealand	-43.87	172.89	South Pacific Ocean	A	
45	Grosser *et al*., 2015	little penguin	*Eudyptula minor*	Stewart Island, New Zealand	-46.85	168.02	South Pacific Ocean	A	
45	Grosser *et al*., 2015	little penguin	*Eudyptula minor*	Oamaru, New Zealand	-44.98	170.98	South Pacific Ocean	B	
45	Grosser *et al*., 2015	little penguin	*Eudyptula minor*	Otago Peninsula, New Zealand	-45.79	170.58	South Pacific Ocean	B	
45	Grosser *et al*., 2015	little penguin	*Eudyptula minor*	Phillip Island, Australia	-38.47	145.2	South Pacific Ocean	B	
45	Grosser *et al*., 2015	little penguin	*Eudyptula minor*	Kangaroo Island, Australia	-35.62	137.6	South Pacific Ocean	B	
45	Grosser *et al*., 2015	little penguin	*Eudyptula minor*	Pearson, Australia	-32.33	133.72	South Pacific Ocean	B	
45	Grosser *et al*., 2015	little penguin	*Eudyptula minor*	Cheyne, Australia	-34.96	118.2	South Pacific Ocean	B	2
46	Ritchie *et al*., 2004	adélie penguin	*Pygoscelis adeliae*	Welsh Island, Antarctica	-67.33	62.75	Indian Ocean	A	
46	Ritchie *et al*., 2004	adélie penguin	*Pygoscelis adeliae*	Gardner Island, Antarctica	-68.34	77.52	Indian Ocean	A	
46	Ritchie *et al*., 2004	adélie penguin	*Pygoscelis adeliae*	Torgersen Island, Antarctica	-64.46	64.5	South Atlantic Ocean	A	1
46	Ritchie *et al*., 2004	adélie penguin	*Pygoscelis adeliae*	Cape bird, Ross Island, Antarctica	-77.14	166.28	South Pacific Ocean	B	
46	Ritchie *et al*., 2004	adélie penguin	*Pygoscelis adeliae*	Cape Royds, Ross Island, Antarctica	-77.33	166.1	South Pacific Ocean	B	
46	Ritchie *et al*., 2004	adélie penguin	*Pygoscelis adeliae*	Cape Crozier, Ross island, Antarctica	-77.3	169.22	South Pacific Ocean	B	
46	Ritchie *et al*., 2004	adélie penguin	*Pygoscelis adeliae*	Beaufort Island, Antarctica	-76.56	167.3	South Pacific Ocean	B	
46	Ritchie *et al*., 2004	adélie penguin	*Pygoscelis adeliae*	Franklin Island, Antarctica	-76.5	168.19	South Pacific Ocean	B	
46	Ritchie *et al*., 2004	adélie penguin	*Pygoscelis adeliae*	Inexpressible Island, Antarctica	-74.79	165.5	South Pacific Ocean	B	
46	Ritchie *et al*., 2004	adélie penguin	*Pygoscelis adeliae*	Adélie Cove, Antarctica	-74.76	165.4	South Pacific Ocean	B	
46	Ritchie *et al*., 2004	adélie penguin	*Pygoscelis adeliae*	Edmonson point, Antarctica	-74.73	165.3	South Pacific Ocean	B	
46	Ritchie *et al*., 2004	adélie penguin	*Pygoscelis adeliae*	Cape Wheatstone, Antarctica	-72.17	170.14	South Pacific Ocean	B	
46	Ritchie *et al*., 2004	adélie penguin	*Pygoscelis adeliae*	Cape Hallet, Antarctica	-72.19	170.16	South Pacific Ocean	B	
46	Ritchie *et al*., 2004	adélie penguin	*Pygoscelis adeliae*	Cape Adare, Antarctica	-72.2	170.84	South Pacific Ocean	B	
46	Ritchie *et al*., 2004	adélie penguin	*Pygoscelis adeliae*	Port Martin, Antarctica	-68.46	162.96	South Pacific Ocean	B	2
46	Ritchie *et al*., 2004	adélie penguin	*Pygoscelis adeliae*	Balleny Islands, Antarctica	-66.73	162.53	South Pacific Ocean	B	
47	Clucas *et al*., 2014	chinstrap penguin	*Pygoscelis antarctica*	Orne Harbour, Antarctica	-65.7	-64.48	Antarctic peninsula	A	1
47	Clucas *et al*., 2014	chinstrap penguin	*Pygoscelis antarctica*	King Georges, South Shetlands	-61.7	-58	Antarctic	A	
47	Clucas *et al*., 2014	chinstrap penguin	*Pygoscelis antarctica*	Signy Island, Orkney Island	-60.19	-46.25	Antarctic	A	
47	Clucas *et al*., 2014	chinstrap penguin	*Pygoscelis antarctica*	Thula, South Sandwiches Island	-59.44	-27.42	Antarctic	B	2
48	Clucas *et al*., 2014	gentoo penguin	*Pygoscelis papua*	Volunteer Point, Falkland Islands	-51.45	-58.05	South Atlantic Ocean	A	1

48	Clucas *et al*., 2014	gentoo penguin	*Pygoscelis papua*	Saunders, Falkland Islands	-51.24	60.08	South Atlantic Ocean	A	
48	Clucas *et al*., 2014	gentoo penguin	*Pygoscelis papua*	Bird Island, South Georgia	-54	-38.03	South Atlantic Ocean	B	
48	Clucas *et al*., 2014	gentoo penguin	*Pygoscelis papua*	South Orkneys, Signy	-60.41	-45.6	South Atlantic Ocean	C	
48	Clucas *et al*., 2014	gentoo penguin	*Pygoscelis papua*	South Shetlands, King Georges	-61.99	-58.01	Antarctic	C	
48	Clucas *et al*., 2014	gentoo penguin	*Pygoscelis papua*	Port Lockroy	-64.88	-63.53	Antarctic	C	2
49	Bouzat *et al*., 2009	magellanic penguin	*Spheniscus magellanicus*	Caleta Valdes, N. Patagonia, Argentina	-42.28	-63.21	South Atlantic Ocean	A	1
49	Bouzat *et al*., 2009	magellanic penguin	*Spheniscus magellanicus*	Punta Tombo, N Patagonia, Argentina	-44.02	-65.11	South Atlantic Ocean	A	
49	Bouzat *et al*., 2009	magellanic penguin	*Spheniscus magellanicus*	Cabo Dos Bahias, N Patagonia, Argentina	-44.54	-65.32	South Atlantic Ocean	A	
49	Bouzat *et al*., 2009	magellanic penguin	*Spheniscus magellanicus*	Puerto Deseado, S. Patagonia, Argentina	-47.45	-65.56	South Atlantic Ocean	B	
49	Bouzat *et al*., 2009	magellanic penguin	*Spheniscus magellanicus*	Punta Quilla, S. Patagonia, Argentina	-50.07	-68.23	South Atlantic Ocean	B	
49	Bouzat *et al*., 2009	magellanic penguin	*Spheniscus magellanicus*	Cabo Virgenes, S. Patagonia, Argentina	-52.2	-68.21	South Atlantic Ocean	B	
49	Bouzat *et al*., 2009	magellanic penguin	*Spheniscus magellanicus*	New-Island, Falkland Islands	-51.42	-61.16	South Atlantic Ocean	C	
49	Bouzat *et al*., 2009	magellanic penguin	*Spheniscus magellanicus*	Volunteer point, Falkland Islands	-51.28	-57.5	South Atlantic Ocean	C	2
49	Bouzat *et al*., 2009	magellanic penguin	*Spheniscus magellanicus*	Seal bay, Falkland Islands	-51.24	-58.02	South Atlantic Ocean	C	1
50	Sonsthagen *et al*., 2012	European herring gull	*Larus argentatus*	Iceland[δ]	66.04	-17.95	North Atlantic Ocean	A	1
50	Sonsthagen *et al*., 2012	European herring gull	*Larus argentatus*	Tromso, Norway[δ]	69.67	18.96	North Atlantic Ocean	B	
50	Sonsthagen *et al*., 2012	European herring gull	*Larus argentatus*	Finistere, Ile de Balanec France[δ]	48.42	-4.98	North Atlantic Ocean	A	
50	Sonsthagen *et al*., 2012	European herring gull	*Larus argentatus*	Tyumenskaya Oblast, Russia[δ]	56.19	68.95	North Atlantic Ocean	C	
50	Sonsthagen *et al*., 2012	European herring gull	*Larus argentatus*	Chukoskiy Avtonomny Okrug, Russia[δ]	65.48	177.58	North Atlantic Ocean	C	2
50	Sonsthagen *et al*., 2012	European herring gull	*Larus argentatus*	New York, US[δ]	40.57	-73.99	North Atlantic Ocean	D	
50	Sonsthagen *et al*., 2012	European herring gull	*Larus argentatus*	Great Slave Lake, Northwest territories, Canada[δ]	61.61	-115.25	Middle land	D	
50	Sonsthagen *et al*., 2012	European herring gull	*Larus argentatus*	Prince Edward Island, Canada[δ]	45.5	-62.35	North Atlantic Ocean	D	
50	Sonsthagen *et al*., 2012	European herring gull	*Larus argentatus*	Minnesota, US[δ]	48.06	-93.68	North Atlantic Ocean	D	
50	Sonsthagen *et al*., 2012	European herring gull	*Larus argentatus*	Maryland, US[δ]	38.74	-76.42	North Atlantic Ocean	D	
50	Sonsthagen *et al*., 2012	European herring gull	*Larus argentatus*	Alaska, US[δ]	55.19	-152.31	North Atlantic Ocean	D	
51	Liebers *et al*., 2001	yellow-legged gull	*Larus cachinnans*	Azores, Portugal[δ]	37.77	-25.8	North Atlantic Ocean	A	1
51	Liebers *et al*., 2001	yellow-legged gull	*Larus cachinnans*	Madeira, Portugal[δ]	33.34	-16.96	North Atlantic Ocean	A	
51	Liebers *et al*., 2001	yellow-legged gull	*Larus cachinnans*	Morocco[δ]	30.36	-10.43	North Atlantic Ocean	A	
51	Liebers *et al*., 2001	yellow-legged gull	*Larus cachinnans*	Berlenga, Portugal[δ]	39.41	-9.51	North Atlantic Ocean	A	
51	Liebers *et al*., 2001	yellow-legged gull	*Larus cachinnans*	Galicia, Spain[δ]	42.92	-8.01	North Atlantic Ocean	A	
51	Liebers *et al*., 2001	yellow-legged gull	*Larus cachinnans*	Gibraltar, Portugal[δ]	36.109	-5.34	North Atlantic Ocean	A	
51	Liebers *et al*., 2001	yellow-legged gull	*Larus cachinnans*	Camargue, France[δ]	43.56	4.55	North Atlantic Ocean	A	
51	Liebers *et al*., 2001	yellow-legged gull	*Larus cachinnans*	Alsace, France[δ]	48.159	7.6	Mediterranean	A	
51	Liebers *et al*., 2001	yellow-legged gull	*Larus cachinnans*	Northwest Italy[δ]	41.51	12.54	Mediterranean	A	
51	Liebers *et al*., 2001	yellow-legged gull	*Larus cachinnans*	Malta, Portugal[δ]	36.08	14.42	Mediterranean	A	
51	Liebers *et al*., 2001	yellow-legged gull	*Larus cachinnans*	Crete[δ]	35.24	24.56	Mediterranean	A	
51	Liebers *et al*., 2001	yellow-legged gull	*Larus cachinnans*	Constanta, Romania[δ]	44.2	28.64	Mediterranean	A	
51	Liebers *et al*., 2001	yellow-legged gull	*Larus cachinnans*	Tuz Golu, Turkey[δ]	38.87	33.38	Aralo-Caspian region	A	
51	Liebers *et al*., 2001	yellow-legged gull	*Larus cachinnans*	Van Gulu, Turkey[δ]	38.62	42.9	Aralo-Caspian region	A	

51	Liebers *et al*., 2001	yellow-legged gull	*Larus cachinnans*	Beysehir Golu, Turkey[δ]	37.77	31.51	Aralo-Caspian region	A		
51	Liebers *et al*., 2001	yellow-legged gull	*Larus cachinnans*	Istria, Romania[δ]	44.59	28.71	Aralo-Caspian region	B		
51	Liebers *et al*., 2001	yellow-legged gull	*Larus cachinnans*	Odessa, Ukraine[δ]	46.48	30.76	Aralo-Caspian region	B		
51	Liebers *et al*., 2001	yellow-legged gull	*Larus cachinnans*	Asov Sea, Ukraine[δ]	46.17	36.58	Aralo-Caspian region	B		
51	Liebers *et al*., 2001	yellow-legged gull	*Larus cachinnans*	Caspian Sea, Russia[δ]	42.61	50.71	Aralo-Caspian region	B		
51	Liebers *et al*., 2001	yellow-legged gull	*Larus cachinnans*	Tengiz, Kazakhstan[δ]	50.24	68.8	Aralo-Caspian region	B		
51	Liebers *et al*., 2001	yellow-legged gull	*Larus cachinnans*	Tuzkan, Uzbekistan[δ]	40.59	67.41	Aralo-Caspian region	B		
51	Liebers *et al*., 2001	yellow-legged gull	*Larus cachinnans*	Eastern Mongolia[δ]	48.4	115.23	Aralo-Caspian region	B		
51	Liebers *et al*., 2001	yellow-legged gull	*Larus cachinnans*	Lake Baikal, Russia[δ]	53.59	108.15	Aralo-Caspian region	B		
51	Liebers *et al*., 2001	yellow-legged gull	*Larus cachinnans*	Western Mongolia[δ]	47.2	98.2	Aralo-Caspian region	B		2
52	Sonsthagen *et al*., 2012	common gull	*Larus canus*	Sweden	62.48	14.03	North Atlantic Ocean	A		1
52	Sonsthagen *et al*., 2012	common gull	*Larus canus*	Kamchatka, Russia	57.96	161.95	Sea of Okhotsk North Pacific Ocean	A		2
52	Sonsthagen *et al*., 2012	common gull	*Larus canus*	South Central Alaska, US	62.32	-145.39	North Pacific Ocean	B		
52	Sonsthagen *et al*., 2012	common gull	*Larus canus*	Central Alaska, US	64.97	-148.73	North Pacific Ocean	B		
52	Sonsthagen *et al*., 2012	common gull	*Larus canus*	Great Slave Lake, Northwest territories, Canada	61.61	-115.25	Middle land	B		
53	Liebers & Helbig, 2002	lesser black-backed gull	*Larus fuscus*	Iceland	64.09	-21.57	North Atlantic Ocean	A		
53	Liebers & Helbig, 2002	lesser black-backed gull	*Larus fuscus*	Faroe Islands	62	-7	North Atlantic Ocean	A		
53	Liebers & Helbig, 2002	lesser black-backed gull	*Larus fuscus*	Northern England	53.46	-2.42	North Atlantic Ocean	A		
53	Liebers & Helbig, 2002	lesser black-backed gull	*Larus fuscus*	Central England	53.25	-2.1	North Atlantic Ocean	A		1
53	Liebers & Helbig, 2002	lesser black-backed gull	*Larus fuscus*	Rotterdam, The Netherlands	51.55	4.28	North Atlantic Ocean	A		
53	Liebers & Helbig, 2002	lesser black-backed gull	*Larus fuscus*	Finistere, France	48.2	-4	North Atlantic Ocean	A		
53	Liebers & Helbig, 2002	lesser black-backed gull	*Larus fuscus*	Vest-Adger, Norway	58.2	6.4	North Atlantic Ocean	A		
53	Liebers & Helbig, 2002	lesser black-backed gull	*Larus fuscus*	Germany, North Sea	54.4	8.2	North Atlantic Ocean	A		
53	Liebers & Helbig, 2002	lesser black-backed gull	*Larus fuscus*	Salthom, Denmark	55.4	12.45	North Atlantic Ocean	A		
53	Liebers & Helbig, 2002	lesser black-backed gull	*Larus fuscus*	Vaasa, Finland	63.06	21.36	Barents Sea	B		
53	Liebers & Helbig, 2002	lesser black-backed gull	*Larus fuscus*	Tampere, Finland	61.3	23.45	Barents Sea	B		
53	Liebers & Helbig, 2002	lesser black-backed gull	*Larus fuscus*	Helsinki, Finland	60.1	24.48	Barents Sea	B		
53	Liebers & Helbig, 2002	lesser black-backed gull	*Larus fuscus*	Kuopio, Finland	62.54	27.41	Barents Sea	B		
53	Liebers & Helbig, 2002	lesser black-backed gull	*Larus fuscus*	Savonlina, Finland	61.52	28.53	Barents Sea	B		
53	Liebers & Helbig, 2002	lesser black-backed gull	*Larus fuscus*	Lake Saimaa, Finland	61.15	28.15	Barents Sea	B		
53	Liebers & Helbig, 2002	lesser black-backed gull	*Larus fuscus*	White Sea, Russia	66.35	32.45	Barents Sea	B		
53	Liebers & Helbig, 2002	lesser black-backed gull	*Larus fuscus*	Finnish Bay, Russia	59.4	28.2	Barents Sea	B		
53	Liebers & Helbig, 2002	lesser black-backed gull	*Larus fuscus*	West Siberia	67.4	44.1	Barents Sea	C		
53	Liebers & Helbig, 2002	lesser black-backed gull	*Larus fuscus*	Kanin Peninsula, Russia	67.2	44.1	Barents Sea	C		
53	Liebers & Helbig, 2002	lesser black-backed gull	*Larus fuscus*	Petchora, Delta, Russia	67	52.3	Barents Sea	C		
53	Liebers & Helbig, 2002	lesser black-backed gull	*Larus fuscus*	Pur District, Russia	65.3	77.3	Kara sea	C		
53	Liebers & Helbig, 2002	lesser black-backed gull	*Larus fuscus*	Taimyr Peninsula, Russia	74.1	86.3	Kara Sea	C		2
54	Sonsthagen *et al*., 2012	glaucus-winged gull	*Larus glaucescens*	Washington, US	38.9	-78.93	North Pacific Ocean	A		1
54	Sonsthagen *et al*., 2012	glaucus-winged gull	*Larus glaucescens*	Oregon, US	46.29	-124.35	North Pacific Ocean	A		

54	Sonsthagen *et al*., 2012	glaucus-winged gull	*Larus glaucescens*	Kachemak Bay, Homer, Alaska, US	59.48	-151.47	North Pacific Ocean	A	
54	Sonsthagen *et al*., 2012	glaucus-winged gull	*Larus glaucescens*	Middelton Island, Alaska, US	59.42	-146.33	North Pacific Ocean	A	
54	Sonsthagen *et al*., 2012	glaucus-winged gull	*Larus glaucescens*	Aleutians Island, Alaska, US	52.17	-172.49	North Pacific Ocean	A	
54	Sonsthagen *et al*., 2012	glaucus-winged gull	*Larus glaucescens*	Vancouver Island, British Columbia, Canada	49.47	-126.59	North Pacific Ocean	A	
54	Sonsthagen *et al*., 2012	glaucus-winged gull	*Larus glaucescens*	Queen Charlotte Island, British Columbia, Canada	53.28	-132.03	North Pacific Ocean	A	2
55	Sonsthagen *et al*., 2012	glaucus gull	*Larus hyperboreus*	Bjarnarhafnarfjall, Iceland	65.48	-20.59	North Atlantic Ocean	A	1
55	Sonsthagen *et al*., 2012	glaucus gull	*Larus hyperboreus*	Scoresbysund, Greenland	77.93	-21.82	North Atlantic Ocean	B	
55	Sonsthagen *et al*., 2012	glaucus gull	*Larus hyperboreus*	Svalbard Arch, Spitsbergen, Norway	77.26	14.21	North Atlantic Ocean	B	
55	Sonsthagen *et al*., 2012	glaucus gull	*Larus hyperboreus*	Yukon-Kuskokwim, Alaska, US	59.99	-163.64	Arctic Ocean	B	2
55	Sonsthagen *et al*., 2012	glaucus gull	*Larus hyperboreus*	Baffin Island, Canada	64.01	-68.5	North Atlantic Ocean	B	
55	Sonsthagen *et al*., 2012	glaucus gull	*Larus hyperboreus*	North Slope Borough, Alaska, US	68.1	-165.71	Arctic Ocean	B	
56	Pons *et al*., 2013	great black-backed gulls	*Larus marinus*	New York, JFK airport, US	40.63	-73.8	North Atlantic Ocean	A	
56	Pons *et al*., 2013	great black-backed gulls	*Larus marinus*	North Carolina, US	35.34	-76.27	North Atlantic Ocean	A	1
56	Pons *et al*., 2013	great black-backed gulls	*Larus marinus*	Virginia, Wachreague Inlet, US	38.2	-75.77	North Atlantic Ocean	A	
56	Pons *et al*., 2013	great black-backed gulls	*Larus marinus*	Missouri, Smithville, US	39.73	-94.75	North Atlantic Ocean	A	
56	Pons *et al*., 2013	great black-backed gulls	*Larus marinus*	Maine, US	44.26	-68.66	North Atlantic Ocean	A	
56	Pons *et al*., 2013	great black-backed gulls	*Larus marinus*	Louisiana, US	29.52	-91.6	Gulf of Mexico	A	
56	Pons *et al*., 2013	great black-backed gulls	*Larus marinus*	Massachussets, US	42.66	-71.36	North Atlantic Ocean	A	
56	Pons *et al*., 2013	great black-backed gulls	*Larus marinus*	Maryland, US	37.99	-75.68	North Atlantic Ocean	A	
56	Pons *et al*., 2013	great black-backed gulls	*Larus marinus*	Quebec, Canada	45.47	-73.34	North Atlantic Ocean	A	
56	Pons *et al*., 2013	great black-backed gulls	*Larus marinus*	Brittany, France	47.82	-4.39	North Atlantic Ocean	B	
56	Pons *et al*., 2013	great black-backed gulls	*Larus marinus*	Faroe Islands	62.06	-7	North Atlantic Ocean	B	
56	Pons *et al*., 2013	great black-backed gulls	*Larus marinus*	Denmark	57.15	9.02	North Atlantic Ocean	B	
56	Pons *et al*., 2013	great black-backed gulls	*Larus marinus*	Finland	61.26	21.18	Barents Sea	B	2
56	Pons *et al*., 2013	great black-backed gulls	*Larus marinus*	Sweden	55.74	12.88	North Atlantic Ocean	B	
57	Patirana *et al*., 2002	red-legged kittiwake	*Rissa brevirostris*	Bering Island	55.12	166.21	Bering Sea	A	1
57	Patirana *et al*., 2002	red-legged kittiwake	*Rissa brevirostris*	Buldir Island, Alaska US	53.09	175.74	North Pacific Ocean	B	
57	Patirana *et al*., 2002	red-legged kittiwake	*Rissa brevirostris*	Saint Georges Island, Florida US	57.49	-170.29	North Pacific Ocean	B	2
58	Yeung *et al*., 2009	white Tern	*Gygis alba*	Northern Mariana Islands	15.18	145.72	Central Pacific	A	1
58	Yeung *et al*., 2009	white Tern	*Gygis alba*	Marshall Islands	7.34	169.21	Central Pacific	A	
58	Yeung *et al*., 2009	white Tern	*Gygis alba*	Kiritimati Island	1.95	-157.36	Central Pacific	A	
58	Yeung *et al*., 2009	white Tern	*Gygis alba*	Northwestern Hawaiian Islands, US	28.42	-178.32	North Pacific Ocean	A	
58	Yeung *et al*., 2009	white Tern	*Gygis alba*	Oahu Island, Hawaii, US	21.48	-157.98	North Pacific Ocean	A	
58	Yeung *et al*., 2009	white Tern	*Gygis alba*	Marquesas Islands	-9.73	-139.08	South Pacific Ocean	B	
58	Yeung *et al*., 2009	white Tern	*Gygis alba*	Tuamotu Archipelago, French Polynesia	-15.99	-145.64	South Pacific Ocean	B	2
59	Faria *et al*., 2010	American tern	*Sterna hirundinacea*	Escalvada Island, Brazil	-20.42	-40.24	South Atlantic Ocean	A	1
59	Faria *et al*., 2010	American tern	*Sterna hirundinacea*	Itacuce Island, Brazil	-23.5	-45.26	South Atlantic Ocean	A	
59	Faria *et al*., 2010	American tern	*Sterna hirundinacea*	Apara Island, Brazil	-23.49	-45.32	South Atlantic Ocean	A	
59	Faria *et al*., 2010	American tern	*Sterna hirundinacea*	Laje de Santos, Brazil	-24.19	-46.11	South Atlantic Ocean	A	

59	Faria *et al*., 2010	American tern	*Sterna hirundinacea*	Cardos Island, Brazil	-27.48	-48.34	South Atlantic Ocean	A		
59	Faria *et al*., 2010	American tern	*Sterna hirundinacea*	Chubut, Argentinian Patagonia region	-42.49	-64.28	South Atlantic Ocean	B	2	
60	Miller *et al*., 2013	gull-billed tern	*Gelochelidon nilotica*	South Bay Salt Works, California, US	32.6	-117.09	North Pacific Ocean	A	1	
60	Miller *et al*., 2013	gull-billed tern	*Gelochelidon nilotica*	Texas, US	27.3	-97.43	Gulf of Mexico	A		
60	Miller *et al*., 2013	gull-billed tern	*Gelochelidon nilotica*	Virginia, US	37.6	-75.63	North Atlantic Ocean	A	2	
61	Draheim *et al*., 2010	least tern	*Sterna antillarum*	Alameda, California, US	37.78	-122.25	Pacific Ocean	A	1	
61	Draheim *et al*., 2010	least tern	*Sterna antillarum*	San Diego, California, US	32.72	-117.15	Pacific Ocean	A		
61	Draheim *et al*., 2010	least tern	*Sterna antillarum*	Mc clean, North Dakota, US	48.01	-100.91	Inland US	A		
61	Draheim *et al*., 2010	least tern	*Sterna antillarum*	Yankton, North Dakota, US	46.75	-98.71	Inland US	A		
61	Draheim *et al*., 2010	least tern	*Sterna antillarum*	Pottawatomie, Kansas, US	39.38	-96.35	Inland US	A		
61	Draheim *et al*., 2010	least tern	*Sterna antillarum*	New Madrid, Missouri, US	36.59	-89.52	Inland US	A		
61	Draheim *et al*., 2010	least tern	*Sterna antillarum*	Woods, Oklahoma, US	36.76	-98.71	Inland US	A		
61	Draheim *et al*., 2010	least tern	*Sterna antillarum*	Tulsa, Oklahoma, US	36.29	-95.98	Inland US	A		
61	Draheim *et al*., 2010	least tern	*Sterna antillarum*	Mccurtain, Oklahoma, US	35.16	-94.96	Inland US	A		
61	Draheim *et al*., 2010	least tern	*Sterna antillarum*	Dallas, Texas, US	32.8	-96.78	Inland US	A		
61	Draheim *et al*., 2010	least tern	*Sterna antillarum*	Bolivar, Mississippi, US	33.66	-91.05	Inland US	A		
61	Draheim *et al*., 2010	least tern	*Sterna antillarum*	Knox, Maine, US	44.54	-69.22	Atlantic Ocean	A	2	
61	Draheim *et al*., 2010	least tern	*Sterna antillarum*	Barnstable, Massachussets, US	41.72	-70.3	Atlantic Ocean	A		
61	Draheim *et al*., 2010	least tern	*Sterna antillarum*	Cape May, New Jersey, US	38.93	-74.9	Atlantic Ocean	A		
61	Draheim *et al*., 2010	least tern	*Sterna antillarum*	Accomack, Virginia, US	38.07	-75.65	Atlantic Ocean	A		
61	Draheim *et al*., 2010	least tern	*Sterna antillarum*	Glenn, Virginia, US	38.57	-77.59	Atlantic Ocean	A		
61	Draheim *et al*., 2010	least tern	*Sterna antillarum*	St. Croix, Virginia, US	36.84	-76.13	Atlantic Ocean	A		
61	Draheim *et al*., 2010	least tern	*Sterna antillarum*	Bay, Florida, US	30.3	-85.55	Atlantic Ocean	A		
61	Draheim *et al*., 2010	least tern	*Sterna antillarum*	Harrisson, Mississippi, US	30.52	-89	Atlantic Ocean	A		
61	Draheim *et al*., 2010	least tern	*Sterna antillarum*	Brazoria, Texas, US	29.05	-95.56	Atlantic Ocean	A		
62	Avise *et al*., 2000	sooty tern$^{\Psi}$	*Sterna fuscata*	Chagos archipelagos	-6.12	72.14	Indian Ocean	A		
62	Avise *et al*., 2000	sooty tern$^{\Psi}$	*Sterna fuscata*	Seychelles	-4.65	55.43	Indian Ocean	A	1	
62	Avise *et al*., 2000	sooty tern$^{\Psi}$	*Sterna fuscata*	Johnston Island	16.72	-169.53	South Pacific Ocean	A	2	
62	Avise *et al*., 2000	sooty tern$^{\Psi}$	*Sterna fuscata*	Puerto Rico	18.508	-66.38	Caribbean sea	B		
62	Avise *et al*., 2000	sooty tern$^{\Psi}$	*Sterna fuscata*	Ascension Island	-7.93	-14.35	South Atlantic Ocean	B		
63	Pshenichnikova *et al*., 2015	crested auklet	*Aethia cristatella*	Talan Island, Russia	59.2	149.3	Sea of Okhotsk	A	1	
63	Pshenichnikova *et al*., 2015	crested auklet	*Aethia cristatella*	Saint Jonah Island, Russia	56.24	143.22	Sea of Okhotsk	A		
63	Pshenichnikova *et al*., 2015	crested auklet	*Aethia cristatella*	Medny Island, Russia	54.4	167.5	Bering Sea	A		
63	Pshenichnikova *et al*., 2015	crested auklet	*Aethia cristatella*	Kamchatka Island, Russia	57.42	169.32	Bering Sea	A		
63	Pshenichnikova *et al*., 2015	crested auklet	*Aethia cristatella*	Kuril Area, Russia	47.25	154.28	Sea of Okhotsk	A	2	
64	Pshenichnikova *et al*., 2017	whiskered auklet	*Aethia pygmaea*	Saint Jonah Island, Russia	56.24	143.23	Sea of Okhotsk	A	1	
64	Pshenichnikova *et al*., 2017	whiskered auklet	*Aethia pygmaea*	Kuril Area, Russia	48.17	153.15	Sea of Okhotsk	A		
64	Pshenichnikova *et al*., 2017	whiskered auklet	*Aethia pygmaea*	Commander Island, Russia	55	166.15	Bering Sea	B		
64	Pshenichnikova *et al*., 2017	whiskered auklet	*Aethia pygmaea*	Buldir Island, Aleutian Islands, US	52.21	175.55	Bering Sea	C	2	

65	Moum & Arnason, 2001	razorbill	*Alca torda*	Quebec, Canada	50.19	-59.39	North Atlantic Ocean	A	1
65	Moum & Arnason, 2001	razorbill	*Alca torda*	Gannet Islands, Newfoundland, Canada	54	-56.3	North Atlantic Ocean	A	
65	Moum & Arnason, 2001	razorbill	*Alca torda*	Latrabjarg, Iceland	65.2	-24.3	East Atlantic	B	
65	Moum & Arnason, 2001	razorbill	*Alca torda*	The Baltic, UK	60.15	19.27	East Atlantic	C	
65	Moum & Arnason, 2001	razorbill	*Alca torda*	Hornoya, Norway	72.22	31.1	East Atlantic	C	2
66	Wojczulanis-Jakubas *et al*., 2015	little auk	*Alle alle*	Paakitsoq fjord, Thule district, Northwest Greenland, US	76.16	-68.57	North Atlantic Ocean	A	1
66	Wojczulanis-Jakubas *et al*., 2015	little auk	*Alle alle*	Hornsund, southwest Spitzbergen, Svalbar	77	15.33	North Atlantic Ocean	A	
66	Wojczulanis-Jakubas *et al*., 2015	little auk	*Alle alle*	Tikhaya Bay, Hooker Island, Franz Joseph land, Russia	80.18	52.49	North Atlantic Ocean	A	2
67	Birt *et al*., 2011	kittlitz's murrelet	*Brachyramphus brevirostris*	Attu Island, Western Aleutian Islands, US	52.89	173.08	North Pacific Ocean	A	1
67	Birt *et al*., 2011	kittlitz's murrelet	*Brachyramphus brevirostris*	Kachemak Bay, Alaska, US	59.55	-151.32	North Pacific Ocean	B	
67	Birt *et al*., 2011	kittlitz's murrelet	*Brachyramphus brevirostris*	Glacier Bay, Alaska, US	58.12	-134.08	North Pacific Ocean	B	2
68	Friesen *et al*., 1996	marbled murrelet	*Brachyramphus marmoratus*	Magadan, Russia[δ,]	59.78	150.29	Sea of Okkhotsk, North Pacific Ocean	A	1
68	Friesen *et al*., 1996	marbled murrelet	*Brachyramphus marmoratus*	Attu, Alaska[δ,]	53.35	172.93	Bering Sea	B	
68	Friesen *et al*., 1996	marbled murrelet	*Brachyramphus marmoratus*	Mitrofania, Alaska[δ,]	56.06	-158.72	North Pacific Ocean	B	
68	Friesen *et al*., 1996	marbled murrelet	*Brachyramphus marmoratus*	Kodiak, Alaska[δ,]	57.82	-152.53	North Pacific Ocean	B	
68	Friesen *et al*., 1996	marbled murrelet	*Brachyramphus marmoratus*	Kachemak, Alaska[δ,]	59.72	-151.52	North Pacific Ocean	B	
68	Friesen *et al*., 1996	marbled murrelet	*Brachyramphus marmoratus*	Unakwik, Alaska[δ,]	61.12	-147.58	North Pacific Ocean	B	
68	Friesen *et al*., 1996	marbled murrelet	*Brachyramphus marmoratus*	Yakutat, Alaska[δ,]	59.54	-140.43	Gulf of Alaska	B	
68	Friesen *et al*., 1996	marbled murrelet	*Brachyramphus marmoratus*	Humboldt, WA[δ,]	47.37	-124.32	North Pacific Ocean	B	
68	Friesen *et al*., 1996	marbled murrelet	*Brachyramphus marmoratus*	Southwest Oregon[δ,]	43.37	-124.45	North Pacific Ocean	B	2
69	Wallace *et al*., 2014	cassin's auklet	*Ptychoramphus aleuticus*	Buldir Island, Aleutian Islands, US	52.36	175.02	North Pacific Ocean	A	1
69	Wallace *et al*., 2014	cassin's auklet	*Ptychoramphus aleuticus*	Ulak Island, Aleutian Islands, US	51.51	-178.47	North Pacific Ocean	A	
69	Wallace *et al*., 2014	cassin's auklet	*Ptychoramphus aleuticus*	Adak Island, Aleutian Islands, US	51.41	-176.18	North Pacific Ocean	A	
69	Wallace *et al*., 2014	cassin's auklet	*Ptychoramphus aleuticus*	Chugidanak Island, Aleutian Islands, US	52.28	-169.2	North Pacific Ocean	A	
69	Wallace *et al*., 2014	cassin's auklet	*Ptychoramphus aleuticus*	Kagamil Island, Aleutian Islands, US	52.59	-169.42	North Pacific Ocean	A	
69	Wallace *et al*., 2014	cassin's auklet	*Ptychoramphus aleuticus*	Aitkak Island, Aleutian Islands, US	54.57	-164.58	North Pacific Ocean	A	
69	Wallace *et al*., 2014	cassin's auklet	*Ptychoramphus aleuticus*	Big & Little Koniuji Island, Aleutian Islands, US	55.57	-159.59	North Pacific Ocean	A	
69	Wallace *et al*., 2014	cassin's auklet	*Ptychoramphus aleuticus*	Semidi Island, Aleutian Islands, US	56.52	-156.01	North Pacific Ocean	A	
69	Wallace *et al*., 2014	cassin's auklet	*Ptychoramphus aleuticus*	Lowrie Island, Southeast Alaska, US	54.51	-133.32	North Pacific Ocean	A	
69	Wallace *et al*., 2014	cassin's auklet	*Ptychoramphus aleuticus*	Frederick Island, British Columbia, Canada	53.55	-133.09	North Pacific Ocean	A	
69	Wallace *et al*., 2014	cassin's auklet	*Ptychoramphus aleuticus*	Reef Island, British Columbia, Canada	52.51	-131.31	North Pacific Ocean	A	
69	Wallace *et al*., 2014	cassin's auklet	*Ptychoramphus aleuticus*	Triangle Island, British Columbia, Canada	50.51	-129.05	North Pacific Ocean	A	
69	Wallace *et al*., 2014	cassin's auklet	*Ptychoramphus aleuticus*	Washington Coast, Washington, US	46.02	-123.55	North Pacific Ocean	A	
69	Wallace *et al*., 2014	cassin's auklet	*Ptychoramphus aleuticus*	Southeast Farallon Island, California, US	37.41	-123	North Pacific Ocean	A	
69	Wallace *et al*., 2014	cassin's auklet	*Ptychoramphus aleuticus*	Channel Island, California, US	34.15	-120.2	North Pacific Ocean	B	
69	Wallace *et al*., 2014	cassin's auklet	*Ptychoramphus aleuticus*	San Jeronimo Island, Baja California, US	29.51	-115.48	North Pacific Ocean	B	
69	Wallace *et al*., 2014	cassin's auklet	*Ptychoramphus aleuticus*	Guadalupe Island, Baja California, US	29.13	-118.23	North Pacific Ocean	B	
69	Wallace *et al*., 2014	cassin's auklet	*Ptychoramphus aleuticus*	San Benito Island, Baja California, US	28.18	-115.34	North Pacific Ocean	B	2

70	Pearce *et al*., 2002	ancient murrelet	*Synthliboramphus antiquus*	Kamchatka peninsula, Russia	51.24	157.29	West North Pacific Ocean	A	1
70	Pearce *et al*., 2002	ancient murrelet	*Synthliboramphus antiquus*	Kurile Islands, Russia	47.8	152.99	West North Pacific Ocean	A	
70	Pearce *et al*., 2002	ancient murrelet	*Synthliboramphus antiquus*	East Limestone Island, British Columbia, Canada	52.9	-131.62	East Pacific Ocean	A	
70	Pearce *et al*., 2002	ancient murrelet	*Synthliboramphus antiquus*	George Island, British Columbia, Canada	52.35	-131.18	East Pacific Ocean	A	2
71	Birt *et al*., 2011	xantus's murrelet	*Synthliboramphus hypoleucus*	San Miguel, California, US[δ,]	34.03	-120.38	North Pacific Ocean	A	
71	Birt *et al*., 2011	xantus's murrelet	*Synthliboramphus hypoleucus*	Santa Cruz, California, US[δ,]	33.97	-119.74	North Pacific Ocean	A	1
71	Birt *et al*., 2011	xantus's murrelet	*Synthliboramphus hypoleucus*	Anacapa, California, US[δ,]	34.02	-119.52	North Pacific Ocean	A	
71	Birt *et al*., 2011	xantus's murrelet	*Synthliboramphus hypoleucus*	Santa Barbara, California, US[δ,]	33.33	-118.45	North Pacific Ocean	A	
71	Birt *et al*., 2011	xantus's murrelet	*Synthliboramphus hypoleucus*	Santa Catalina, California, US[δ,]	33.32	-118.33	North Pacific Ocean	A	
71	Birt *et al*., 2011	xantus's murrelet	*Synthliboramphus hypoleucus*	San Clemente, California, US[δ,]	32.84	-118.48	North Pacific Ocean	A	
71	Birt *et al*., 2011	xantus's murrelet	*Synthliboramphus hypoleucus*	Coronado, California, US[δ,]	32.69	-117.189	North Pacific Ocean	A	
71	Birt *et al*., 2011	xantus's murrelet	*Synthliboramphus hypoleucus*	Todos Santos, California, US[δ,]	31.83	-116.69	North Pacific Ocean	A	
71	Birt *et al*., 2011	xantus's murrelet	*Synthliboramphus hypoleucus*	San Martin, Mexico[δ,]	30.44	-116.06	North Pacific Ocean	A	
71	Birt *et al*., 2011	xantus's murrelet	*Synthliboramphus hypoleucus*	San Jeronimo, Mexico[δ,]	29.85	-115.72	North Pacific Ocean	A	
71	Birt *et al*., 2011	xantus's murrelet	*Synthliboramphus hypoleucus*	Cedros, Mexico[δ,]	28.04	-115.28	North Pacific Ocean	A	
71	Birt *et al*., 2011	xantus's murrelet	*Synthliboramphus hypoleucus*	San Benito, Mexico[δ,]	28.31	-115.58	North Pacific Ocean	A	2
71	Birt *et al*., 2011	xantus's murrelet	*Synthliboramphus hypoleucus*	Guadalupe, Mexico[δ,]	28.91	-118.28	North Pacific Ocean	B	
72	Morris-Pocock *et al*., 2008	common guillemot	*Uria aalge*	Witless Bay, Newfoundland, Canada	47.16	-52.49	North Atlantic Ocean	A	
72	Morris-Pocock *et al*., 2008	common guillemot	*Uria aalge*	Gannet Islands, Newfoundland, Canada	53.56	-56.32	North Atlantic Ocean	A	
72	Morris-Pocock *et al*., 2008	common guillemot	*Uria aalge*	Funk Island, Newfoundland, Canada	40.45	-53.11	North Atlantic Ocean	A	
72	Morris-Pocock *et al*., 2008	common guillemot	*Uria aalge*	Latrabjarg, Iceland	65.38	-24.19	North Atlantic Ocean	B	
72	Morris-Pocock *et al*., 2008	common guillemot	*Uria aalge*	Shetlands Islands, Scotland	60.19	-1.25	North Atlantic Ocean	B	
72	Morris-Pocock *et al*., 2008	common guillemot	*Uria aalge*	Isle of May, Scotland	56.11	-2.33	North Atlantic Ocean	B	
72	Morris-Pocock *et al*., 2008	common guillemot	*Uria aalge*	Runde, Norway	62.24	5.39	North Atlantic Ocean	B	
72	Morris-Pocock *et al*., 2008	common guillemot	*Uria aalge*	Bornholm, Denmark	55.37	14.35	Baltic Sea	B	
72	Morris-Pocock *et al*., 2008	common guillemot	*Uria aalge*	Skomer Island, Wales	51.45	-5.11	North Atlantic Ocean	B	
72	Morris-Pocock *et al*., 2008	common guillemot	*Uria aalge*	Hornoya, Norway	70.22	31.8	Arctic Ocean	C	
72	Morris-Pocock *et al*., 2008	common guillemot	*Uria aalge*	Bjornoya, Norway	74.26	19.21	Arctic Ocean	C	
72	Morris-Pocock *et al*., 2008	common guillemot	*Uria aalge*	Novaya Zemlya, Russia	74.23	56.25	Arctic Ocean	C	1
72	Morris-Pocock *et al*., 2008	common guillemot	*Uria aalge*	Sea of Okhotsk, eastern Russia	58.55	169.47	Sea of Okkhotsk, North Pacific Ocean	D	
72	Morris-Pocock *et al*., 2008	common guillemot	*Uria aalge*	Cape Lisburne, Chuckchi Sea, US	68.52	-166.14	Arctic Ocean	D	
72	Morris-Pocock *et al*., 2008	common guillemot	*Uria aalge*	Fairway Rock, Bering Strait	65.4	-168.58	Arctic Ocean	D	
72	Morris-Pocock *et al*., 2008	common guillemot	*Uria aalge*	Pribilof Island, Bering Sea, US	56.53	-169.59	Arctic Ocean	D	
72	Morris-Pocock *et al*., 2008	common guillemot	*Uria aalge*	Attu Island, Western Aleutian Islands, US	52.55	-172.26	North Pacific Ocean	D	
72	Morris-Pocock *et al*., 2008	common guillemot	*Uria aalge*	Bogoslof Island, central Aleutian Islands, US	53.56	-168.03	North Pacific Ocean	D	
72	Morris-Pocock *et al*., 2008	common guillemot	*Uria aalge*	Aiktak Island, Eastern Aleutian Islands, US	54.11	-164.5	North Pacific Ocean	D	
72	Morris-Pocock *et al*., 2008	common guillemot	*Uria aalge*	Midun Island, Western Alaskan peninsula, US	54.51	-162.11	North Pacific Ocean	D	
72	Morris-Pocock *et al*., 2008	common guillemot	*Uria aalge*	Big Koniuji Island, Central Alaskan peninsula, US	55.05	-159.3	North Pacific Ocean	D	

72	Morris-Pocock *et al*., 2008	common guillemot	*Uria aalge*	Chowiet Island, Eastern Alaskan peninsula, US	56.04	-156.42	North Pacific Ocean	D		
72	Morris-Pocock *et al*., 2008	common guillemot	*Uria aalge*	East Amatuli Island, Lower Cook inlet, US	58.53	-152	North Pacific Ocean	D		
72	Morris-Pocock *et al*., 2008	common guillemot	*Uria aalge*	Chisik Island, Central Cook inlet, US	60.08	-152.33	North Pacific Ocean	D		
72	Morris-Pocock *et al*., 2008	common guillemot	*Uria aalge*	Middleton Island, Prince William sound, Alaska, US	59.26	-146.18	North Pacific Ocean	D		
72	Morris-Pocock *et al*., 2008	common guillemot	*Uria aalge*	Triangle Island, British Columbia, Canada	50.52	-129.05	North Pacific Ocean	D		
72	Morris-Pocock *et al*., 2008	common guillemot	*Uria aalge*	Cape flattery, Washington, US	48.38	-124.71	North Pacific Ocean	D		
72	Morris-Pocock *et al*., 2008	common guillemot	*Uria aalge*	Newport, Oregon, US	44.63	-124.05	North Pacific Ocean	D		
72	Morris-Pocock *et al*., 2008	common guillemot	*Uria aalge*	Farallon Island, Califomia, US	37.7	-123	North Pacific Ocean	D	2	
73	Tigano *et al*., 2015	brünnich's guillemot	*Uria lomvia*	Kamchatka, Russia	52.30	161.56	Sea of Okhotsk	A	1	
73	Tigano *et al*., 2015	brünnich's guillemot	*Uria lomvia*	Buldir Island, Alaska, US	52.20	175.54	Bering Sea	A		
73	Tigano *et al*., 2015	brünnich's guillemot	*Uria lomvia*	Bogoslof Island, Alaska, US	53.55	-168.02	Bering Sea	A		
73	Tigano *et al*., 2015	brünnich's guillemot	*Uria lomvia*	Aiktak, Alaska, US	54.11	-164.51	Bering Sea	A		
73	Tigano *et al*., 2015	brünnich's guillemot	*Uria lomvia*	Pribilof Island, Alaska, US	56.53	-169.59	Bering Sea	A		
73	Tigano *et al*., 2015	brünnich's guillemot	*Uria lomvia*	Cape Lisbume, Alaska, US	68.52	-166.14	Chukchi Sea	A		
73	Tigano *et al*., 2015	brünnich's guillemot	*Uria lomvia*	Cape Thompson, Alaska, US	68.07	-165.53	Chukchi Sea	A		
73	Tigano *et al*., 2015	brünnich's guillemot	*Uria lomvia*	Wrangel Island, Russia	71.02	178.31	Chukchi Sea	B		
73	Tigano *et al*., 2015	brünnich's guillemot	*Uria lomvia*	New Siberian Island, Russia	73.27	142.14	Arctic Ocean	C		
73	Tigano *et al*., 2015	brünnich's guillemot	*Uria lomvia*	Hornøya, Norway	72.22	31.10	North Atlantic Ocean	D		
73	Tigano *et al*., 2015	brünnich's guillemot	*Uria lomvia*	Látrapjag, Iceland	65.38	-24.19	North Atlantic Ocean	D		
73	Tigano *et al*., 2015	brünnich's guillemot	*Uria lomvia*	Kipako, Greenland, US	73.42	-56.38	North Atlantic Ocean	D		
73	Tigano *et al*., 2015	brünnich's guillemot	*Uria lomvia*	Hakluyt Island, Greenland, US	77.26	-72.40	North Atlantic Ocean	D		
73	Tigano *et al*., 2015	brünnich's guillemot	*Uria lomvia*	Coburg Island, Canada	75.48	-79.25	North Atlantic Ocean	D		
73	Tigano *et al*., 2015	brünnich's guillemot	*Uria lomvia*	Gannet Island, Canada	53.42	-56.12	North Atlantic Ocean	D	2	
73	Tigano *et al*., 2015	brünnich's guillemot	*Uria lomvia*	Akpatok Island, Canada	60.32	-68.30	North Atlantic Ocean	D		
73	Tigano *et al*., 2015	brünnich's guillemot	*Uria lomvia*	Digges Island, Canada	62.33	-77.46	North Atlantic Ocean	D		
73	Tigano *et al*., 2015	brünnich's guillemot	*Uria lomvia*	Coats Island, Canada	62.57	-82.00	North Atlantic Ocean	D		
73	Tigano *et al*., 2015	brünnich's guillemot	*Uria lomvia*	Cape Parry, Canada	70.02	-124.07	Arctic Ocean	E		

[δ] seabird colonies currently identified as distinct species as reported in HBW and BirdLife Taxonomic Checklist 2017: **4** = 4· scopoli's shearwater *Calonectris diomedea*, 4· Cory's shearwater *Calonectris borealis*, Gómez-Díaz *et al*., 2009, **6** = 6· northern fulmar *Fulmarus glacialis*, 6· southern fulmar *Fulmarus glacialoides*, Kerr & Dove, 2013, **11** = 11·monteiro's storm petrel *Oceanodroma monteiroi,* 11·cape verde storm petrel *Oceanodroma jabejabe*, Oliveira *et al*., 2013, **22** = 22· zino's petrel *Pterodroma madeira*, 22· cape verde petrel *Pterodroma ferae*, 22· desertas petrel *Pterodroma deserta*, Gangloff *et al*., 2013, **28 =** campbell albatross *Melanophris impavida*, Burg & Croxall, 2001, **44** = 44· southern rockhopper penguin *Eudyptes chrysocome*, 44· northern rockhopper penguin *Eudyptes moseleyi,* Banks *et al*., 2006, **50** = 50· and 50· could not be identified based on the sampled colonies, 50· European herring gull *Larus argentatus*, 50· arctic herring gull *Larus smithsonianus* Sonsthagen *et al*., 2012, **51** = 51· yellow-legged gull *Larus michaellis*, 51· armenian gull *Larus armenicus*, 51· caspian gull *Larus cachinnans*, Liebers *et al*., 2001, **68** = 68· long-billed murrelet *Brachyramphus perdix***,** 68· marbled murrelet *Brachyramphus marmoratus*, Friesen *et al*., 1996**, 71** = 71· scripp's murrelet *Synthliboramphus scrippsi*, 71·guadalupe murrelet *Synthliboramphus hypoleucus*, Chesser *et al*., 2012.

[Ψ]species whose the scientific names have been revised as reported in HBW and Birdlife Taxonomic Checklist 2017: **12** Leach's storm petrel *Hydrobates leucorhous*, Bicknell *et al*., 2012, **13** slender-billed prion *Pachyptila belcheri*, Quillfeldt *et al*., 2017, **37** imperial shag *Leucocarbo atriceps*, Harris *et al*., 2014, **43** yellow-eyed penguin *Megadyptes antipodes*, Boessenkool *et al*., 2009, **62** sooty tern *Onychoprion fuscatus*, Avise *et al*., 2000.

SI. 6 – 2 GenBank accession numbers for 68 seabird species obtained in the literature and used to calculate indices of genetic structure (F_{st}, Φ_{st}), indices of genetic diversity (h, π) and Tajima's D. When the F_{st} , Φ_{st} h, π and Tajima's D could not be calculated based on mtDNA sequences available on GenBank, these indices are reported as obtained in the genetic studies (see Table 6 – 1).

Family	n°	Species	Common name	Marker	bp	GenBank accession numbers	F_{st}
Procellariidae	**1**	*Ardenna carneipes*	flesh-footed shearwater	Cytb	857	KY443815 - KY443957	0.747*
Procellariidae	**2**	*Ardenna mauretanicus*	balearic shearwater	Cytb	886	DQ230222 - DQ230316	0.145*
Procellariidae	**3**	*Ardenna tenuirostris*	short-tailed shearwater	REs	-	-	0.284*
Procellariidae	**4**	*Calonectris diomedea*	Cory's shearwater	CR	293	FJ755483 - FJ755616	0.560*
Procellariidae	**5**	*Diomedea exulans*	wandering albatross	CR	234	AY016127 - AY016173	0.639*
Procellariidae	**6**	*Fulmarus glacialis*	northern fulmar	CR	228	KC755550 - KC755680	0.783*
Procellariidae	**7**	*Halobaena caeruela*	blue petrel	Cytb	889	MF421869 - MF421843	0.008
Procellariidae	**8**	*Hydrobates pelagicus*	European storm petrel	Cytb	910	FJ972203 - AF469071	0.937*
Procellariidae	**9**	*Macronectes giganteus*	southern giant petrel	Cytb	752	GQ120464 - GQ120476	0.572*
Procellariidae	**10**	*Macronectes halli*	northern giant petrel	Cytb	752	GQ120456 - GQ120463	0.207*
Procellariidae	**11**	*Oceanodroma castro*	band-rumped storm petrel	CR	448	AY771004, AY771005, DQ 178703 - DQ 178869	0.730*
Procellariidae	**12**	*Oceanodroma l. leucorhoa*	Leach's storm petrel	CR	357	JQ51396 - JQ513945	0.283*
Procellariidae	**13**	*Pachyptila belcheri*	thin-billed prion	Cytb	889	MF421842 - MF421817	0.326*
Procellariidae	**14**	*Pachyptila desolata*	Antarctic prion	Cytb	889	MF421898 - MF421870	-0.026
Procellariidae	**15**	*Pachyptila turtur*	fairy prion	REs	-	-	0.100
Procellariidae	**16**	*Pelagodroma marina*	white-faced storm-petrel	CR	522	KR109979 - KR110046	0.893*
Procellariidae	**17**	*Phoebastria immutabilis*	Laysan albatross	CR	189	GU395302 - GU395489	0.135*
Procellariidae	**18**	*Phoebastria nigripes*	black-footed albatross	CR	609	AY641399 - AY641403	0.854*
Procellariidae	**19**	*Procellaria aequinoctialis*	white chinned petrel	Cytb	599	EU053409 - EU053425	0.602*
Procellariidae	**20**	*Pterodroma arminjoniana*	Trinidade petrel	Cytb	995	GQ328969 - GQ328972, GQ328974 - GQ328977, GQ328980 - GQ328986	0.265*
Procellariidae	**21**	*Pterodroma cookii*	Cook's petrel	COX1	677	HQ263645 - HQ263663	0.758*

Procellariidae	**22**	*Pterodroma mollis*	gadfly petrel	Cytb	872	JX674282 - JX674488, JX674280 - JX674281, JX674306 - JX674381, JX674450 -JX674461, JX674284 - JX674305, JX674413 - JX674449	0.948*
Procellariidae	**23**	*Pterodroma phaeopygia*	Galápagos petrel	Cytb	1143	HQ420319 - HQ420350	0.044
Procellariidae	**24**	*Pterodroma sandwichensis*	Hawaiian petrel	Cytb	495	DQ 178703 - DQ 178869	0.450*
Procellariidae	**25**	*Pterodroma solandri*	providence petrel	Cytb	872	KX123188 - KX123006	0.022
Procellariidae	**26**	*Thalassarche cauta*	shy albatross	CR	299	FJ617141, FJ617146 - FJ617152, FJ617170 - FJ617176	0.082
Procellariidae	**27**	*Thalassarche chrysostoma*	grey-headed albatross	CR	220	AF326413 - AF326458	0.032
Procellariidae	**28**	*Thalassarche melanophris*	black-browed albatross	CR	211	AY016052 - AY016094, AY016095 - AY016108	0.568*
Procellariidae	**29**	*Thalassarche steadi*	white capped albatross	Cytb	299	FJ617142 - FJ617145, FJ617152 - FJ617169, FJ617177	0.014
Sulidae	**30**	*Sula dactylatra*	masked booby	Cytb	450	AY156695 - AY156699	0.641*
Sulidae	**31**	*Sula grandi*	nazca booby	Cytb/ND2/COI	780	-	-
Sulidae	**32**	*Sula leucogaster*	brown booby	CR	489	GU059603 - GU059720	0.768*
Sulidae	**33**	*Sula nebouxii*	blue-footed booby	CR	538	HQ334115 - HQ334172, HQ334018 - HQ334111	0.047
Sulidae	**34**	*Sula sula*	red-footed booby	CR	473	GU059721 - GU059741, GU059744 - GU059768, GU059771, GU059773-GU059776, GU059778 - GU059779, GU059781- GU059812, GU059838-GU059861	0.736*
Sulidae	**35**	*Sula variegata*	Peruvian booby	CR	540	HQ592377 - HQ592522	0.009
Phalacrocoracidae	**36**	*Phalacrocorax aristotelis*	European cormorant	NADH	320	HM449750 - HM449752	0.937*
Phalacrocoracidae	**37**	*Phalacrocorax atriceps*	imperial shag	ATPase	657	KF983857 - KF983946	0.498*
Phalacrocoracidae	**38**	*Phalacrocorax auritus*	double-crested cormorant	CR	700	KC462062 - KC462148	0.636*
Phalacrocoracidae	**39**	*Phalacrocorax carbo*	great cormorant	CR	434	-	0.131*
Phalacrocoracidae	**40**	*Phalacrocorax magellanicus*	rock shag	ATPase	657	KF983947 - KF984029	0.713*
Sphenicidae	**41**	*Aptenodytes forsteri*	emperor penguin	CR	368	KP644787 - KP645015	0.160*
Sphenicidae	**42**	*Aptenodytes patagonicus*	king penguin	CR	615	KX857217 - KX857259	0.017
Sphenicidae	**43**	*Eudyptes antipodes*	yellow-eyed penguin	CR	731	FJ822137 - FJ822143	0.220*
Sphenicidae	**44**	*Eudyptes chrysocome*	rockhopper penguin	Cytb	668	DQ525741 - DQ525800	0.946*
Sphenicidae	**45**	*Eudyptula minor*	little penguin	CR	387	KP308908 - KP309419	0.815*
Sphenicidae	**46**	*Pygoscelis adeliae*	adélie penguin	CR	594	-	-
Sphenicidae	**47**	*Pygoscelis antarctica*	chinstrap penguin	CR	441	KJ646313 - KJ646148	0.028
Sphenicidae	**48**	*Pygoscelis papua*	gentoo penguin	CR	307	KJ646314 - KJ646562	0.577*
Sphenicidae	**49**	*Spheniscus magellanicus*	magellanic penguin	COI	686	FJ407094 - FJ407180	0.050
Laridae	**50**	*Larus argentatus*	European herring gull	CR	389	JQ709774 - JQ709677	0.215*

Laridae	**51**	*Larus cachinnans*	yellow-legged gull	CR	431	AJ634283 - AJ634299, AJ634317, AJ276944 - AJ276945, AJ277127 - AJ277128, AJ276943, AJ277129 - AJ277133, AJ507739, AJ507752 -AJ507757, AJ507762 - AJ507766, AJ508340, AJ508336, AJ634315- AJ634316, AJ277134, AJ507745, AJ634300 -AJ634307, AJ276942, AJ507746 - AJ507750, AJ507758 - AJ507761, AJ508337 - AJ508339	0.725*
Laridae	**52**	*Larus canus*	common gull	CR	288	JQ709775-JQ709828	0.416*
Laridae	**53**	*Larus fuscus*	lesser black-backed gull	CR	430	AJ634308 - AJ634331, AJ507737 - AJ507744, AJ508341 - AJ508342, AJ277128, AJ277135	0.183
Laridae	**54**	*Larus glaucescens*	glaucus-winged gull	CR	386	JQ709829 - JQ709899	0.125
Laridae	**55**	*Larus hyperboreus*	glaucus gull	CR	392	JQ709981 - JQ709909	0.697*
Laridae	**56**	*Larus marinus*	great Black-backed gull	CR	391	KF422969 - KF422942, AJ276949, AJ508306, AJ508305, AJ508304, AJ276948	0.196*
Laridae	**57**	*Rissa brevirostris*	red-legged kittiwake	CR	35	Haplotypes from Patirana 2002	0.176*
Laridae	**58**	*Gygis alba*	white tern	Cytb	502	EU516389 - EU516525	0.440*
Laridae	**59**	*Sterna hirundinacea*	American tern	NADH	799	EU572709 - EU572716	-0.013
Laridae	**60**	*Gelochelidon nilotica*	gull-billed tern	Cytb	719	KC513400 - KC513418	0.114
Laridae	**61**	*Sterna antillarum*	least tern	CR	840	EU268189 - EU268123	0.138
Laridae	**62**	*Sterna fuscata*	sooty tern	CR	343	AF205605	0.356*
Alcidae	**63**	*Aethia cristatella*	crested auklet	CR	406	KJ409697 - KJ409771	0.020
Alcidae	**64**	*Aethia pygmaea*	whiskered auklet	CR	671	KU891326 - KU891279	0.131*
Alcidae	**65**	*Alca torda*	razorbill	CR	300	AJ410923 - AJ410965, AJ410956	0.034
Alcidae	**66**	*Alle alle*	little auk	CR	481	KM520047 - KM520121	-0.013
Alcidae	**67**	*Brachyramphus brevirostris*	kittlitz's murrelet	CR	330	JN257122 - JN257134	0.935*
Alcidae	**68**	*Brachyramphus marmoratus*	marbled murrelet	Cytb	1044	U63057, U63044 - U63056	0.981*
Alcidae	**69**	*Ptychoramphus aleuticus*	cassin's auklet	CR	649	KF472311 - KF472223	0.304*
Alcidae	**70**	*Synthliboramphus antiquus*	ancient murrelet	CR	1131	AB070632	0.005
Alcidae	**71**	*Synthliboramphus hypoleucus*	xantus's murrelet	CR	409	JN382251 - JN382318	0.692*
Alcidae	**72**	*Uria aalge*	common guillemot	CR	699	FJ374317 - FJ374454	0.614*
Alcidae	**73**	*Uria lomvia*	brünnich's guillemot	CR	742	KJ209504 – KJ209652	0.430*

1 Lombal *et al*., 2018; **2** Genovart *et al*., 2007; **3** Austin *et al*., 1994; **4** Gómez-Díaz *et al*., 2009; **5** Burg and Croxall, 2004; **6** Burg *et al*., 2003; **7** Quillfeldt *et al*., 2017; **8** Cagnon *et al*., 2004; **9** Techow *et al*., 2010; **10** Techow *et al*., 2010; **11** Smith and Friesen, 2007; **12** Bicknell *et al*., 2012; **13** Quillfeldt *et al*., 2017; **14** Quillfeldt *et al*., 2017; **15** Ovenden *et al*., 1991; **16** Silva *et al*., 2015; **17** Young, 2010; **18** Walsh and Edwards, 2005; **19** Techow *et al*., 2009; **20** Brown *et al*., 2010; **21** Rayner *et al*., 2011; **22** Gangloff *et al*., 2013; **23** Welch *et al*., 2011; **24** Wiley *et al*., 2012; **25** Lombal *et al*., 2016; **26** Abbott and Double, 2003; **27** Burg and Croxall, 2001; **28** Burg and

Croxall, 2001; **29** Abbott and Double, 2003; **30** Steeves *et al*., 2003; **31** Levin and Parker, 2012; **32** Morris-Pocock *et al*., 2010; **33** Taylor *et al*., 2011; **34** Morris-Pocock *et al*., 2010; **35** Taylor *et al*., 2011; **36** Barlow *et al*., 2011; **37** Calderón *et al*., 2014; **38** Mercer *et al*., 2013; **39** Marion and Le Gentil, 2006; **40** Calderón *et al*., 2014; **41** Younger *et al*., 2015; **42** Clucas *et al*., 2016; **43** Boessenkool *et al*., 2009; **44** Banks *et al*., 2006; **45** Grosser *et al*., 2015; **46** Ritchie *et al*., 2004; **47** Clucas *et al*., 2014; **48** Clucas *et al*., 2014; **49** Bouzat *et al*., 2009; **50** Sonsthagen *et al*., 2012; **51** Liebers *et al*., 2001; **52** Sonsthagen *et al*., 2012; **53** Liebers and Helbig, 2002; **54** Sonsthagen *et al*., 2012; **55** Sonsthagen *et al*., 2012; **56** Pons *et al*., 2013; **57** Patirana *et al*., 2002; **58** Yeung *et al*., 2009; **59** Faria *et al*., 2010; **60** Miller *et al*., 2013; **61** Draheim *et al*., 2010; **62** Avise *et al*., 2000; **63** Pshenichnikova *et al*., 2015; **64** Pshenichnikova *et al*., 2017; **65** Moum and Arnason, 2001; **66** Wojczulanis-Jakubas *et al*., 2015; **67** Birt *et al*., 2011; **68** Friesen et al.,1996; **69** Wallace *et al*., 2014; **70** Pearce *et al*., 2002 **71** Birt *et al*., 2011; **72** Morris-Pocock *et al*., 2008; **73** Tigano *et al*., 2015.

SI. 6 – 3 Information on differences in breeding phenology, morphology and non-breeding status/non-breeding distributions for 73 species

					Differences in		
Family	n°	Species	Common name	Breeding phenology (Y/N)	Morphology (Y/N)	Non-breeding area (Y/N)	Non-breeding status
Procellariidae	**1**	*Ardenna carneipes*	flesh-footed shearwater	-	Y	N	M
Procellariidae	**2**	*Ardenna mauretanicus*	balearic shearwater	-	Y	-	M
Procellariidae	**3**	*Ardenna tenuirostris*	short-tailed shearwater	N[1]	N	N	M
Procellariidae	**4**	*Calonectris diomedea*	Cory's shearwater	-	Y	Y	M
Procellariidae	**5**	*Diomedea exulans*	wandering albatross	Y[α]	Y[α]	-[α]	M[α]
Procellariidae	**6**	*Fulmarus glacialis*	northern fulmar	Y	Y	Y	M
Procellariidae	**7**	*Halobaena caeruela*	blue petrel	N	N	N	M
Procellariidae	**8**	*Hydrobates pelagicus*	European storm petrel	-	Y	-	M
Procellariidae	**9**	*Macronectes giganteus*	southern giant petrel	-	-	-	M
Procellariidae	**10**	*Macronectes halli*	northern giant petrel	-	-	-	M
Procellariidae	**11**	*Oceanodroma castro*	band-rumped storm petrel	Y	N	-	M
Procellariidae	**12**	*Oceanodroma l. leucorhoa*	Leach's storm petrel	-	-	-	M
Procellariidae	**13**	*Pachyptila belcheri*	thin-billed prion	-	-	N	M
Procellariidae	**14**	*Pachyptila desolata*	Antarctic prion		-	Y	M
Procellariidae	**15**	*Pachyptila turtur*	fairy prion	-	-	-	R
Procellariidae	**16**	*Pelagodroma marina*	white-faced storm-petrel	Y	N	-	M
Procellariidae	**17**	*Phoebastria immutabilis*	Laysan albatross	-	-	-	M
Procellariidae	**18**	*Phoebastria nigripes*	black-footed albatross	-	-	-	M
Procellariidae	**19**	*Procellaria aequinoctialis*	white chinned petrel	-	-	-[β]	M
Procellariidae	**20**	*Pterodroma arminjoniana*	Trindade petrel	N	Y	-	M
Procellariidae	**21**	*Pterodroma cookii*	Cook's petrel	Y	Y	Y	M
Procellariidae	**22**	*Pterodroma mollis*	gadfly petrel	Y	Y	Y	M
Procellariidae	**23**	*Pterodroma phaeopygia*	Galápagos petrel	Y	Y	-	R
Procellariidae	**24**	*Pterodroma sandwichensis*	Hawaiian petrel	N	N	-	M
Procellariidae	**25**	*Pterodroma solandri*	providence petrel	N	N	-	M
Procellariidae	**26**	*Thalassarche cauta*	shy albatross	N	N	-	M

Procellariidae	**27**	*Thalassarche chrysostoma*	grey-headed albatross	N	N	-	M
Procellariidae	**28**	*Thalassarche melanophris*	black-browed albatross	Y	Y	Y	M
Procellariidae	**29**	*Thalassarche steadi*	white capped albatross	-	-	-	M
Sulidae	**30**	*Sula dactylatra*	masked booby	N	Y!	-	R
Sulidae	**31**	*Sula grandi*	nazca booby	N	-	-	R
Sulidae	**32**	*Sula leucogaster*	brown booby	N	Y	-	R
Sulidae	**33**	*Sula nebouxii*	blue-footed booby	N	N	-	R
Sulidae	**34**	*Sula sula*	red-footed booby	N	Y	-	M
Sulidae	**35**	*Sula variegata*	Peruvian booby	N	N	-	R
Phalacrocoracidae	**36**	*Phalacrocorax aristotelis*	European cormorant	-	Y	-	M$^{\phi}$
Phalacrocoracidae	**37**	*Phalacrocorax atriceps*	imperial shag	-	Y	-	M
Phalacrocoracidae	**38**	*Phalacrocorax auritus*	double-crested cormorant	-	Y	Y	M$^{\phi}$
Phalacrocoracidae	**39**	*Phalacrocorax carbo*	great cormorant	-	Y	-	M$^{\phi}$
Phalacrocoracidae	**40**	*Phalacrocorax magellanicus*	rock shag	-	Y	-	M$^{\phi}$
Sphenicidae	**41**	*Aptenodytes forsteri*	emperor penguin	-	-	-	M
Sphenicidae	**42**	*Aptenodytes patagonicus*	king penguin	-	-	Y	M
Sphenicidae	**43**	*Eudyptes antipodes*	yellow-eyed penguin	-	-	-	R
Sphenicidae	**44**	*Eudyptes chrysocome*	rockhopper penguin	Y	Y	Y	M
Sphenicidae	**45**	*Eudyptula minor*	little penguin	Y	Y	-	R
Sphenicidae	**46**	*Pygoscelis adeliae*	adélie penguin	Y	-	Y	M
Sphenicidae	**47**	*Pygoscelis antarctica*	chinstrap penguin	-	-	-$^{\varepsilon}$	M
Sphenicidae	**48**	*Pygoscelis papua*	gentoo penguin	Y	Y	-	R
Sphenicidae	**49**	*Spheniscus magellanicus*	magellanic penguin	-	-	N	M
Laridae	**50**	*Larus argentatus*	European herring gull	-	Y	-	M
Laridae	**51**	*Larus cachinnans*	yellow-legged gull	Y	Y	-	M
Laridae	**52**	*Larus canus*	common gull	-	-	-	M
Laridae	**53**	*Larus fuscus*	lesser black-backed gull	Y	Y	Y	M
Laridae	**54**	*Larus glaucescens*	glaucus-winged gull	-	-	-	M
Laridae	**55**	*Larus hyperboreus*	glaucus gull	-	-	-	M$^{\phi}$
Laridae	**56**	*Larus marinus*	great Black-backed gull	-	-	-	M$^{\phi}$
Laridae	**57**	*Rissa brevirostris*	red-legged kittiwake	-	-	-	M

Laridae	**58**	*Gygis alba*	white tern	-	Y	-	R
Laridae	**59**	*Sterna hirundinacea*	American tern	Y	-	N	M
Laridae	**60**	*Gelochelidon nilotica*	gull-billed tern	-	Y	Y	M
Laridae	**61**	*Sterna antillarum*	least tern	-	Y	-	M
Laridae	**62**	*Sterna fuscata*	sooty tern	Y	Y!!	-	M
Alcid	**63**	*Aethia cristatella*	crested auklet	N	N	-	M
Alcid	**64**	*Aethia pygmaea*	whiskered auklet	-	Y	-	R
Alcid	**65**	*Alca torda*	razorbill	-	Y	-	M
Alcid	**66**	*Alle alle*	little auk	-	Y	N	M^{ϕ}
Alcid	**67**	*Brachyramphus brevirostris*	kittlitz's murrelet	-	-	-	M^{ϕ}
Alcid	**68**	*Brachyramphus marmoratus*	marbled murrelet	Y	Y	-	M^{ϕ}
Alcid	**69**	*Ptychoramphus aleuticus*	cassin's auklet	Y	Y	-	M^{ϕ}
Alcid	**70**	*Synthliboramphus antiquus*	ancient murrelet	-	-	Y	M
Alcid	**71**	*Synthliboramphus hypoleucus*	xantus's murrelet	Y	Y	-	M^{ϕ}
Alcid	**72**	*Uria aalge*	common guillemot	-	Y	-	M
Alcid	**73**	*Uria lomvia*	brünnich's guillemot	-	Y	-	M^{ϕ}

M4a: M = Migratory species; M^{ϕ} = Partially-migratory species; R = Year resident species
M4b: $M^{\vee}$= Migratory species showing different non-breeding areas among colonies; R = Year resident species; $M^{\wedge}$= Migratory species with overlapping non-breeding areas
M4c: $M^{\vee}$= Migratory species showing different non-breeding areas among colonies; $M^{\wedge}$= Migratory species with overlapping non-breeding areas

$^{\alpha}$Variation in phenotypic traits only observed between colonies separated by the barrier 5a in Figure 1; variation in non-breeding areas between two colonies genetically undifferentiated such as Crozet and Kerguelen Islands (Weimerskirch *et al*., 2015)

$^{\beta}$Variation in non-breeding areas between colonies genetically undifferentiated such as Crozet vs. South Georgia (Weimerskirch *et al*., 1999)

$^{\varepsilon}$Variation in non-breeding areas between two breeding colonies on South Shetland Islands in Antarctica genetically undifferentiated (Trivelpiece *et al*., 2007)

! Variation in morphological traits between genetically undifferentiated Pacific colonies

!! Variation in morphological traits between genetically undifferentiated Indo-Pacific colonies

$^{\delta}$Population-specific non-breeding behavior

1 Hindwood, 1945; Lombal *et al*., 2018 **2** Genovart *et al*., 2007, 2012**;** Guilford *et al*., 2012 **3** Austin *et al*., 1994; Weimerskirch and Cherel, 1998 **4** González-Solís *et al*., 2007; Gómez-Díaz *et al*., 2009; Dias *et al*., 2011 **5** Burg and Croxall, 2004 **6** Weimerskirch *et al*., 2001; Mallory and Forbes, 2007; Hatch *et al*., 2010 **7** Navarro *et al*., 2013; Quillfeldt *et al*., 2017 **8** Wojczulanis-Jakubas and Jensen, 2014; Medeiros *et al*., 2012 **9** Blanco and Quintana, 2014 **10** Warham, 1990 **11** Monteiro and Furness, 1998; Smith and Friesen, 2007; see additional references on the variation in phenology among colonies in Friesen *et al*., 2007; Deane 2013 unpublished **12** Pollet *et al*., 2014 **13 & 14** Cherel *et al*., 2002; Quillfeldt *et al*., 2017 **15** Del Hoyo *et al*., 1992 **16** Silva *et al*., 2015 **17** & **18** Fischer *et al*., 2009 **19** Weimerskirch *et al*., 1999; Mackley *et al*., 2011 **20** Brooke and Rowe, 1996; Krüger *et al*., 2016 **21** Rayner *et al*., 2008, 2010 **22** Gangloff *et al*., 2013; Ramírez *et al*., 2013; Ramos *et al*., 2016, 2017 **23** Friesen *et al*., 2006; Welch *et al*., 2011 **24** Wiley *et al*., 2012; Adams and Flora, 2010 **25** Bester, 2003 **26** Abbott and Double, 2003a, 2003b **27 & 28** Prince *et al*., 1994; Burg and Croxall, 2001; Wakefield *et al*., 2011 **29** Petersen *et al*., 2008 **30** Nelson, 1978; O'Brien and Davies, 1990; Pitman and Jehl, 1998 **31** Nelson, 1978 **32** Nelson, 1978;

Morris-Pocock *et al*., 2010 **33** Nelson, 1978; Taylor *et al*., 2011 **34** Steeves *et al*., 2003; Morris-Pocock *et al*., 2010; Burger and Shaffer, 2008 **35** Taylor *et al*., 2011 **36** Barlow *et al*., 2011; Grist *et al*., 2014 **37** Rasmussen, 1994; Calderón *et al*., 2014 **38** Palmer, 1962; Mercer *et al*., 2013**;** Scherr, Bowman and Abraham, 2010 **39** Grémillet, Storch and Peters, 2000; Marion and Le Gentil, 2006; Gienapp and Bregnballe, 2012 **40** Siegel-Causey, 1997 **41** – **42** Scheffer, Bost and Trathan, 2012; Baylis *et al*., 2015 **43** Boessenkool, Austin, *et al*., 2009; Boessenkool, Star, *et al*., 2009 **44** Hull, 1999; Pütz *et al*., 2002, 2003; Jouventin, Cuthbert and Ottvall, 2006 **45** Banks *et al*., 2002; Overeem, 2005 **46** Whitehead, Johnstone and Burton, 1990; Davis, Boersma and Court, 1996; Davis, Harcourt and Bradshaw, 2001; Clarke *et al*., 2003; Dunn, Silk and Trathan, 2011; Lyver *et al*., 2011 **47** Trivelpiece *et al*., 2007 **48** de Dinechin *et al*., 2012; Black, 2016; Vianna *et al*., 2017 **49** Putz, Ingham and Smith, 2000 **50** Liebers, de Knijff and Helbig, 2004; Huettmann and Diamond, 2000 **51** Munilla, 1997; Liebers, Helbig and De Knijff, 2001 **52** Kilpi and Saurola, 1984 **53** Burger, 1996; Liebers and Helbig, 2002 **54** Hatch, Gill and Mulcahy, 2011 **55 & 56** Huettmann and Diamond, 2000 **57** Orben *et al*., 2018 **58** Yeung, Carlon and Conant, 2009 **59** Branco, 2003; Faria *et al*., 2010 **60** Molina and Erwin, 2006 **61** Thompson *et al*., 1997 **62** Peck and Congdon, 2004; Jaquemet *et al*., 2008; Jaeger *et al*., 2017 **63** Gaston, Jones and Lewington, 1998; Schacter, 2017 **64** Pshenichnikova *et al*., 2017; Schacter and Jones, 2018 **65** Salomonsen, 1944; Clarke, Diamond and Chardine, 2010 **66** Wojczulanis-Jakubas *et al*., 2011; Fort *et al*., 2013 **67** – **68** Lougheed *et al*., 2002; Friesen *et al*., 2005; Bertram *et al*., 2016 **69** Wallace *et al*., 2014 **70** Gaston, Carter and Sealy, 1993; Piatt and Gould, 1994; Gaston, Hashimoto and Wilson, 2017 **71** Birt *et al*., 2011 **72** Friesen *et al*., 1996; Montevecchi *et al*., 2012 **73** Gaston, 1984**.**

SI. 6 – 4 Information on whether Northern Temperate species exhibit a sampling range fragmented by land or ice.

Species whose the sampling range is fragmented by land including the Bering Strait and the Strait of Gibraltar are indicate with '1'. The other species are indicated with '0'.

Family	n°	Species	Common name	Region	Land	Location of separation	F.	D
Procellariidae	**2**	*Ardenna mauretanicus*	balearic Shearwater	N	0	All colonies in Mediterranean	0.145*	-0.54
Procellariidae	**4**	*Calonectris diomedea*	Cory's shearwater	N	1	Mediterranean vs. Atlantic Ocean	0.560*	0.10
Procellariidae	**6**	*Fulmarus glacialis*	northern fulmar	N	1	Pacific Ocean vs. Atlantic Ocean	0.783*	0.21
Procellariidae	**8**	*Hydrobates pelagicus*	European storm petrel	N	1	Mediterranean vs. Atlantic Ocean	0.937*	0.70
Procellariidae	**12**	*Oceanodroma l. leucorhoa*	Leach's storm petrel	N	1	Pacific vs. Atlantic Ocean	0.283*	0.14
Procellariidae	**17**	*Phoebastria immutabilis*	Laysan albatross	N	0	All colonies in the Pacific Ocean	0.135*	-0.87
Procellariidae	**18**	*Phoebastria nigripes*	black-footed albatross	N	0	All colonies in the Pacific Ocean	0.854*	0.97
Procellariidae	**20**	*Pterodroma arminjoniana*	Trinidade petrel	N	1	Atlantic vs. Indian Ocean	0.265*	-0.45
Procellariidae	**22**	*Pterodroma mollis*	gadfly petrel	N	0	All colonies in the Atlantic Ocean	0.948*	1.09
Phalacrocoracidae	**36**	*Phalacrocorax aristotelis*	European cormorant	N	1	Mediterranean vs. Atlantic Ocean	0.937*!	1.14
Phalacrocoracidae	**38**	*Phalacrocorax auritus*	double-crested cormorant	N	1	Pacific vs. Atlantic Ocean	0.636*	0.71
Phalacrocoracidae	**39**	*Phalacrocorax carbo*	great cormorant	N	1	Mediterranean vs. Atlantic Ocean	0.131*	-
Laridae	**50**	*Larus argentatus*	European herring gull	N	0	All colonies in the Atlantic Ocean	0.215*	-0.60
Laridae	**51**	*Larus cachinnans*	yellow-legged gull	N	1	Mediterranean vs. Atlantic Ocean	0.725*	0.86
Laridae	**52**	*Larus canus*	common gull	N	1	Pacific vs. Atlantic Ocean	0.416*	-0.25
Laridae	**53**	*Larus fuscus*	lesser black-backed gull	N	0	All colonies in the Atlantic Ocean	0.183	-2.03*
Laridae	**54**	*Larus glaucescens*	glaucus-winged gull	N	0	All colonies in the Pacific Ocean	0.125	-0.36
Laridae	**55**	*Larus hyperboreus*	glaucous gull	N	1	Pacific vs. Atlantic Ocean	0.697*	-0.46
Laridae	**56**	*Larus marinus*	great Black-backed gull	N	0	All colonies in the Atlantic Ocean	0.196*	0.02
Laridae	**57**	*Rissa brevirostris*	red-legged kittiwake	N	0	All colonies in the Pacific Ocean	0.176*	-1.01
Laridae	**60**	*Gelochelidon nilotica*	gull-billed tern	N	1	Pacific vs. Atlantic Ocean	0.114	-0.90
Laridae	**61**	*Sterna antillarum*	least tern	N	1	Pacific vs. Atlantic Ocean	0.138	-1.43
Alcid	**63**	*Aethia cristatella*	crested auklet	N	1	All colonies in the Pacific (Bering)	0.020	-1.74*
Alcid	**64**	*Aethia pygmaea*	whiskered auklet	N	1	All colonies in the Pacific (Bering)	0.131*	-1.73*
Alcid	**65**	*Alca torda*	razorbill	N	0	All colonies in the Atlantic Ocean	0.034	-1.48*
Alcid	**66**	*Alle alle*	little auk	N	0	All colonies in the Atlantic Ocean	-0.013	-1.60*
Alcid	**67**	*Brachyramphus brevirostris*	kittlitz's murrelet	N	1	All colonies in the Pacific (Bering)	0.935*	1.59
Alcid	**68**	*Brachyramphus marmoratus*	marbled murrelet	N	1	All colonies in the Pacific (Bering)	0.981*	-1.14

Alcid	**69**	*Ptychoramphus aleuticus*	cassin's auklet	N	0	All colonies in the Pacific (California)	0.304*	-0.65
Alcid	**70**	*Synthliboramphus antiquus*	ancient murrelet	N	0	All colonies in the Pacific (Bering)	0.005	1.39
Alcid	**71**	*Synthliboramphus hypoleucus*	xantus's murrelet	N	0	All colonies in the Pacific (California	0.692*	-1.15
Alcid	**72**	*Uria aalge*	common guillemot	N	1	Pacific vs. Atlantic Ocean	0.614*	-1.74*
Alcid	**73**	*Uria lomvia*	thick-billed murre	N	1	Pacific vs. Atlantic Ocean	0.543*	-1.20*

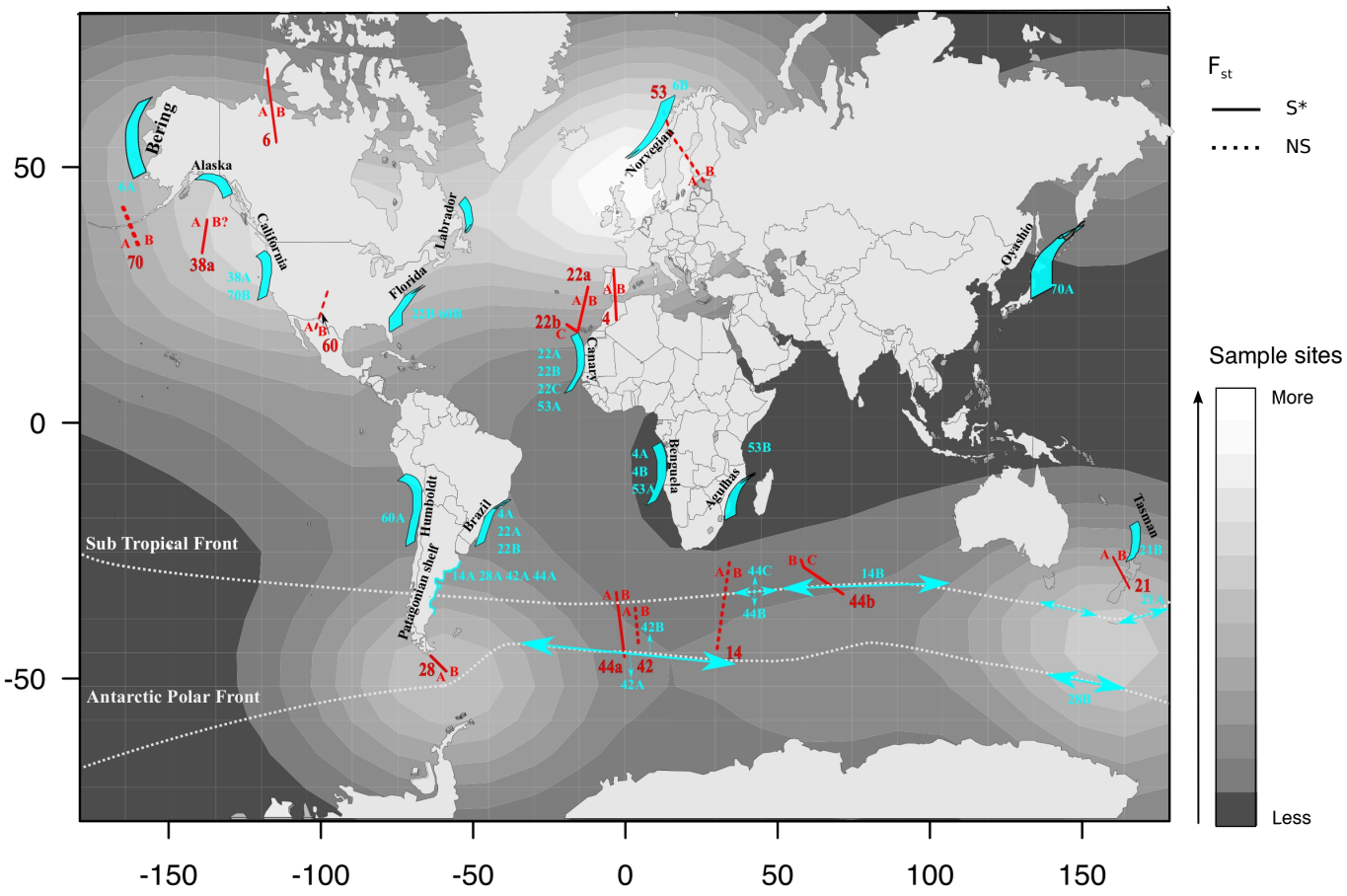
SI. 6 – 5 Information on whether Tropical species exhibit a sampling range fragmented by land. Species whose the sampling range is fragmented by land such as the Isthmus of Panama are indicated with '1'. The other species are indicated with '0'.

Family	n°	Species	Common name	Region	Land	Location of separation	F_{st}	D
Procellariidae	**23**	*Pterodroma phaeopygia*	Galápagos petrel	T	0	All colonies in the Pacific Ocean	0.044	-1.79*
Procellariidae	**24**	*Pterodroma sandwichensis*	Hawaiian petrel	T	0	All colonies in the Pacific Ocean	0.450*	-0.27
Sulidae	**30**	*Sula dactylatra*	masked booby	T	1	Isthmus of Panama	0.641*	1.38
Sulidae	**31**	*Sula grandi*	nazca booby	T	0	All colonies in the Pacific Ocean	-	-
Sulidae	**32**	*Sula leucogaster*	brown booby	T	1	Isthmus of Panama	0.768*	0.88
Sulidae	**33**	*Sula nebouxii*	blue-footed booby	T	0	All colonies in the Pacific Ocean	0.047	-1.40
Sulidae	**34**	*Sula sula*	red-footed booby	T	1	Isthmus of Panama	0.736*	0.06
Sulidae	**35**	*Sula variegata*	Peruvian booby	T	0	All colonies in the Pacific Ocean	0.009	-1.27
Laridae	**58**	*Gygis alba*	white tern	T	0	All colonies in the Pacific Ocean	0.440*	-1.22
Laridae	**62**	*Sterna fuscata*	sooty tern	T	1	Isthmus of Panama	0.356*	-0.71

SI. 6 – 6 Information on whether Southern Temperate species exhibit sample colonies in refuge zones and whether their sampling range cross The Antarctic Polar Front (APF)/Sub-Tropical Front (STF). Species exhibiting sample colonies in refuge zones and species whose the sampling range cross APF/STF are indicated with '1'. The other species are indicated with '0'.

Family	n°	Species	Common name	Region	Sample colonies on refuge zones	Breeding range crossing APF/STF	F_{st}	D
Procellariidae	**1**	*Ardenna carneipes*	flesh-footed shearwater	S	0	0	0.747*	-0.02
Procellariidae	**3**	*Ardenna tenuirostris*	short-tailed shearwater	S	0	0	0.284*	-
Procellariidae	**5**	*Diomedea exulans*	wandering albatross	S	1 Tristan da Cunha, NZ Islands	1	0.639*	-0.57
Procellariidae	**7**	*Halobaena caeruela*	blue petrel	S	0	0	0.008	-1.54
Procellariidae	**9**	*Macronectes giganteus*	southern giant petrel	S	1 Falkland Islands, Gough Island	1	0.572*	0.48
Procellariidae	**10**	*Macronectes halli*	northern giant petrel	S	1 NZ Islands	0	0.207*	-0.46
Procellariidae	**13**	*Pachyptila belcheri*	thin-billed prion	S	1 Falkland Islands	0	0.326*	-1.35
Procellariidae	**14**	*Pachyptila desolata*	Antarctic prion	S	0	0	-0.026	-2.01*
Procellariidae	**15**	*Pachyptila turtur*	fairy prion	S	0	0	0.100	-1.50
Procellariidae	**19**	*Procellaria aequinoctialis*	white chinned petrel	S	1 NZ Islands	0	0.602*	-0.40
Procellariidae	**21**	*Pterodroma cookii*	Cook's petrel	S	0	0	0.758*	0.01
Procellariidae	**25**	*Pterodroma solandri*	providence petrel	S	0	0	0.022	-2.20**
Procellariidae	**26**	*Thalassarche cauta*	shy albatross	S	0	0	0.082	-1.32
Procellariidae	**27**	*Thalassarche chrysostoma*	grey-headed albatross	S	0	1	0.032	-1.32
Procellariidae	**28**	*Thalassarche melanophris*	black-browed albatross	S	1 Falkland Islands	0	0.568*	-0.74
Procellariidae	**29**	*Thalassarche steadi*	white-capped albatross	S	0	0	0.014	-1.60
Phalacrocoracidae	**37**	*Phalacrocorax atriceps*	imperial shag	S	1 Falkland Islands	0	0.498*	0.72
Phalacrocoracidae	**40**	*Phalacrocorax magellanicus*	rock shag	S	1 Falkland Islands	0	0.713*	0.53
Sphenicidae	**41**	*Aptenodytes forsteri*	emperor penguin	S	0	0	0.160*	-0.64
Sphenicidae	**42**	*Aptenodytes patagonicus*	king penguin	S	1 Falkland Islands	0	0.017	1.42
Sphenicidae	**43**	*Eudyptes antipodes*	yellow-eyed penguin	S	0	0	0.220*	-0.13
Sphenicidae	**44**	*Eudyptes chrysocome*	rockhopper penguin	S	1 Falkland Islands, Amsterdam Island	1	0.946*	0.88
Sphenicidae	**45**	*Eudyptula minor*	little penguin	S	0	0	0.815*	3.29**

Sphenicidae	**46**	*Pygoscelis adeliae*	adélie penguin	S	0	0	-	-1.71*
Sphenicidae	**47**	*Pygoscelis antarctica*	chinstrap penguin	S	0	0	0.028	-1.87*
Sphenicidae	**48**	*Pygoscelis papua*	gentoo penguin	S	1 Falkland Islands	1	0.577*	-0.88
Sphenicidae	**49**	*Spheniscus magellanicus*	magellanic penguin	S	1 Falkland Islands	0	0.050	-2.25**
Laridae	**59**	*Sterna hirundinacea*	American tern	S	0	0	-0.013	-0.23



SI. 6 – 7 Non-breeding areas of 13 seabird species for which colonies within species exhibit population-specific distribution during the non-breeding season. Red: continuous lines = locations of genetic barriers for eight seabird species exhibiting a significant F_{st} and population-specific distribution during the non-breeding season ; dotted lines = locations of five species exhibiting a non-significant F_{st} and population-specific distribution during the non-breeding season. The latest are based on the Monmonier's algorithm but do not represent genetic barriers. In blue: locations of non-breeding areas for seabird colonies from each side of the sampling range. **4 Cory's shearwater**: Atlantic (A) and Mediterranean (B) birds move in the Berlenga current (González-Solís *et al.*, 2007) but (A) also move to Brazil currents (Dias *et al.*, 2011), **6 northern Fulmar**: Pacific colonies (A) move towards the Bering current (Hatch *et al.*, 2010) whereas Atlantic colonies (B) migrate to the Norwegian current

(Weimerskirch *et al*., 2001), **14 Antarctic prion**: South Georgia colonies (A) winters on the Patagonian shelf (Quillfeldt *et al*., 2017) whereas Kerguelen birds winters along the Sub-Tropical Front >1000km from the breeding site (Cherel *et al*., 2002), **21 Cook's petrel**: Codfish Islands' colony (A) migrates to the sub-tropical front and Humboldt current whereas Little barrier Island's colony migrates to California and North Pacific currents (Rayner *et al*., 2008), **22 gadfly petrel:** birds from Madeira (A) and Buglio Island (B) exploit area between Canaries and Azores and Brazil current during the non-breeding season (Ramos *et al*., 2016) and (B) also exploits Florida currents further from the colony (Ramírez *et al*., 2013) whereas birds from Cape Verde (C) remains around Cape Verde archipelagos (Ramos *et al*., 2016), **28 black-browed albatross**: birds from Falkland Island (A) migrate to the Patagonian shelf (Wakefield *et al*., 2011) whereas birds from South Georgia (B) move along the Antarctic polar Front (APF) zone south of Africa and Australia during the non-breeding season (Prince *et al*., 1994), **38 double-crested cormorant**: birds from the North Pacific (A) winter in California currents (Mercer *et al*., 2013) whereas birds from interior lands (B) are year-round residents, **42 king penguin:** Falkland island's colony move to the Patagonian shelf (Baylis *et al*., 2015) Birds from South Georgia (A) move south of the APF (Scheffer *et al*., 2012)**,** birds from Possession island and Macquarie island (B) move to the Antarctic Polar Frontal Zone (APFZ) during the non-breeding season**, 44 rockhopper penguin:** Falkland Island's colony (A) winters in the Patagonian shelf (Pütz *et al*., 2002), birds from Crozet and Kerguelen (B) winter south of Sub-Tropical Front and APFZ (Hull, 1999) whereas birds from Amsterdam Island (C) winter north of the Sub-Tropical Front (Jouventin *et al*., 2006), **46 adélie penguin**: not shown on map, winter migrations differ among colonies from Ross sea (Davis *et al*., 2001), Antarctic Peninsula (Dunn *et al*., 2011) and eastern colonies (Clarke *et al*., 2003), **53 lesser black-backed gull**: birds from Finland (B) are long distance migrants and winter along the Agulhas current whereas birds from Norway, Germany and Denmark (A) winter along

the west African coast in the Benguela current (Kilpi and Saurola, 1984), **60 gull-billed tern**: birds from the Atlantic coast (B) winter along Florida whereas birds from the Pacific coast (A) winter in La Paz, Baja California (Molina and Erwin, 2006), **70 ancient murrelet**: Asian populations (A) winter off the coast of Hokkaido (Piatt and Gould, 1994) whereas Canadian murrelets (B) winter off North America (Gaston *et al*., 1993).

SI. 6 – 8 *ad hoc* tests of neutrality for little penguin colonies and genetic clusters

Sampled colony	Sampling size (n)	Tajima's D	Fu and Li's D
Auckland, New Zealand	34	-0.46	1.03
Bay of Plenty, New Zealand	39	-0.42	0.50
Hawke's Bay, New Zealand	9	0.27	0.99
Wellington, New Zealand	14	-1.23	-1.66
Golden Bay, New Zealand	22	-0.07	-0.19
West Coast, New Zealand	40	-1.50	**1.80****
Kaikura, New Zealand	12	**-1.8***	**-2.18***
Chatham Island, New Zealand	8	0.93	1.11
Banks Peninsula, New Zealand	40	-1.46	**-2.31***
Stewart Island, New Zealand	29	-0.53	-0.05
Genetic cluster A	247	-0.80	1.35
Oamaru, New Zealand	96	-1.14	**2.05****
Otago Peninsula, New Zealand	56	0.77	1.23
Phillip Island, Australia	20	0.43	0.34
Kangaroo Island, Australia	20	-0.11	-0.18
Pearson, Australia	10	-0.07	-0.39
Cheyne, Australia	16	-0.6	-0.34
Genetic cluster B	216	**-1.67***	**1.89****

*p<0.05, **p<0.02

SI. 9 *ad hoc* tests of neutrality for whiskered auklet colonies and genetic clusters

Sampled colony	Sampling size (n)	Tajima's D	Fu and Li's D
Saint Jonah Island, Russia	8	-0.88	-0.96
Kuril area, Russia	5	-0.41	-0.41
Genetic cluster A	13	-1.24	-0.92
Commander Island, Russia	19	-0.74	0.20
Genetic cluster B	19	-0.74	0.20
Buldir Island, Aleutian Islands, US	25	**-1.49***	-1.34
Genetic cluster C	25	**-1.49**	-1.34

*p<0.05

SI. 6 – 10 *ad hoc* tests of neutrality for common guillemot colonies and genetic clusters

Sampled colony	Sampling size (n)	Tajima's D	Fu and Li's D
Witless Bay, Newfoundland, Canada	27	**-1.79***	**-2.68***
Gannet Island, Newfoundland, Canada	19	-0.23	0.79
Funk Island, Newfoundland, Canada	27	-0.62	0.11
Genetic cluster A	73	-1.27	-0.71
Latrabjarg, Iceland	19	-1.26	-1.36
Shetlands Island, Scotland	26	**-1.60***	-1.67
Isla of May, Scotland	31	-1.47	-1.68
Runde, Norway	3	_$^{\delta}$	_$^{\delta}$
Bornholm, Denmark	18	-0.11	0.25
Skomer Island, Wales	27	-1.06	-0.34
Genetic cluster B	127	**-1.94***	**-2.68***
Hornoya, Norway	29	-1.35	-1.19
Bjornoya, Norway	10	-0.56	0.031
Novaya Zemlya, Russia	5	-0.80	-0.80
Genetic cluster C	44	**-1.59***	-1.39
Sea of Okhotsk, eastern Russia	24	-0.96	-1.05
Cape Lisburne, Chukchi Sea	26	**-1.59***	**-2.00***
Fairway Rock, Bering Strait, US	2	_$^{\delta}$	_$^{\delta}$
Pribilof Island, Bering Sea, US	32	-1.44	**-2.19***
Attu Island, US	13	-0.42	-0.51
Bogoslof Island, US	6	-1.14	-1.15
Aiktak Island, US	27	**-2.11***	**-3.32****
Midun Island, US	7	-0.65	-0.51
Big Koniuji Island, US	10	**-1.78***	**-2.03***
Chowiet Island, US	17	-1.56	**-2.41***
East Amatuli Island, US	22	**-1.94***	**-2.26***
Chisik Island, US	43	**-2.35****	**-3.90****
Middelton Island, US	20	-1.34	-0.44
Triangle Island, Canada	20	-0.95	-0.85
Cape Flattery, US	11	-0.84	-0.71
Newport, US	21	-1.43	-1.49
Farallon Island, US	21	-1.47	-1.53
Genetic cluster D	322	**-2.45*****	**-5.26****

*p<0.05, **p<0.01, ***p<0.001, $^{\delta}$Tajima's D and Fu and Li's D require a minimum of 4 individuals

SI. 6 – 11 *ad hoc* tests of neutrality for brünnich's guillemot colonies and genetic clusters

Sampled colony	Sampling size (n)	Tajima's D	Fu and Li's D
Kamchatka, Russia	44	-0.87	-0.69
Buldir Island, US	19	-0.96	-0.56
Bogoslof Island, US	13	0.19	-0.04
Aiktak, US	13	-1.29	-1.68
Pribilof Island, US	16	-0.86	-0.80
Cape Lisburne, US	29	0.91	-0.02
Cape Thompson, US	10	0.92	0.57
Genetic cluster A	144	-0.86	**-1.85***
Wrangel Island, Russia	7	0.99	0.63
Genetic cluster B	7	0.99	0.63
New Siberian Island, Russia	5	0.46	0.46
Genetic cluster C	5	0.46	0.46
Hornoya, Norway	28	-1.22	**-2.20***
Látrapjag, Iceland	29	-0.68	-1.03
Kipako, Greenland, US	13	-0.08	-0.61
Hakluyt Island, Greenland, US	14	-1.08	-1.24
Coburg Island, Canada	19	-0.06	-0.29
Gannet Island, Canada	19	**-1.45***	**-2.16***
Akpatok Island, Canada	26	-0.99	-1.14
Digges Island, Canada	26	-0.93	-1.33
Coats Island, Canada	18	-0.77	-1.11
Genetic cluster D	213	**-1.59***	**-2.09***
Cape Parry, Canada	21	-0.55	0.24
Genetic cluster E	21	-0.55	0.24

*$p<0.05$

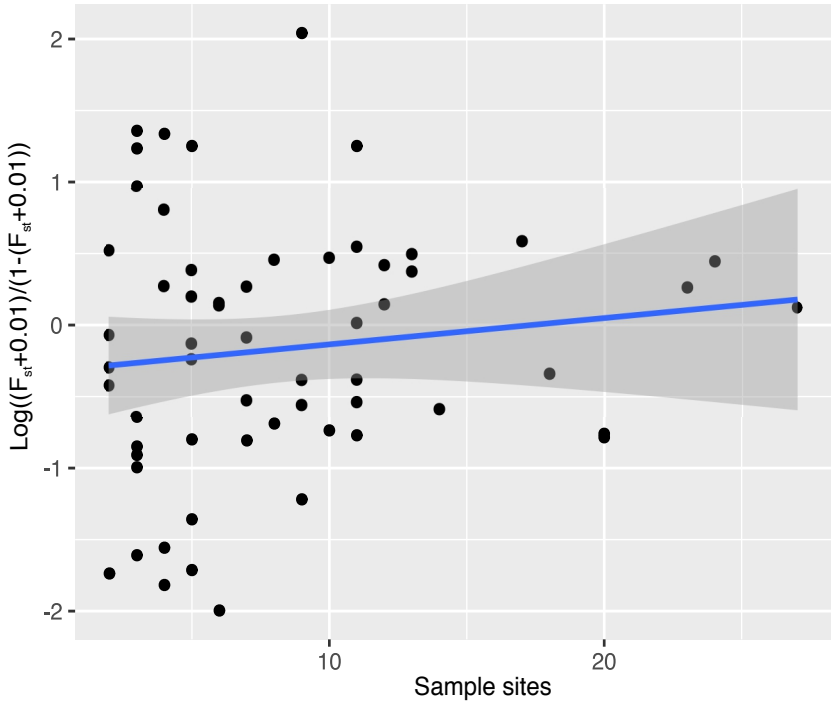
SI. 6 – 12 Generalized linear models M1 – M4a–c where the AIC for all single predictors tested are shown. Results of GLMs for the global set of data (M1) based on i) 70 species for which Tajima's D could be calculated or obtained in the literature and ii) (M1') 59 species in relation to mutation-drift equilibrium (Total number of species = 73 species - 14 species showing significant Tajima's D = 59 species). Additional models M2 – M3 – M4a–c and M5 based on 24, 36, 59, 23, 14 and 22 species for which information on breeding phenology, morphology and non-breeding distributions were available respectively.

		Variables in specific model		χ^2		AIC	
Degree of freedom	Group of models	F_{st} as binomial error distribution	F_{st} as normal error distribution	Binomial	Normal	Binomial	Normal
All species (d.f. = 70)	Single factor	M1 D***	D***	**0.001**	**0.003**	81.44	185.46
With respect to mutation-drift equilibrium (d.f. = 59)	Single factor	M1' Sample sites*	Sample sites	**0.040**	0.406	62.04	157.30
	Single factor	M1' IUCN Status	IUCN Status	0.223	0.788	66.54	161.93
	Single factor	M1' Family	Family	0.572	0.616	70.38	161.41
	Single factor	M1' Population size	Population size	0.530	0.599	65.83	157.64
	Single factor	M1' Sample size (n)	Sample size (n)	0.060	0.521	61.18	157.66
	Single factor	M1' Regions (N/S/T)	Regions	0.383	0.264	67.17	156.81
	Single factor	M1' Distance	Distance	0.216	0.337	64.69	156.99
	Single factor	M1' Genetic marker	Genetic marker	0.438	0.598	69.40	161.25
	Single factor	M1' π	π	0.728	0.893	65.09	154.43
	Single factor	M1' h	h	0.591	0.184	64.92	152.17
With respect to mutation-drift equilibrium (d.f. = 24)	Single factor	M2 Differences in phenology (P)	Differences in phenology (P)*	0.056	**0.024**	29.45	78.94
With respect to mutation-drift equilibrium (d.f. = 36)	Single factor	M3 Differences in morphology (M)*	Differences in morphology (M)	**0.007**	0.058	33.57	97.64
With respect to mutation-drift equilibrium (d.f. = 59)	Single factor	M4a Differences in movement patterns ($M/M^{\phi}/R$)	M4a Differences in movement patterns ($M/M^{\phi}/R$)	0.056	0.100	62.44	153.91
With respect to mutation-drift equilibrium (d.f. = 23)	Single factor	M4b Differences in wintering areas ($(M^{t}+R)/M^{s}$)	Differences in wintering areas ($(M^{t}+R)/M^{s}$)	0.858	0.592	32.23	70.08
With respect to mutation-drift equilibrium (d.f. = 14)	Single factor	M4c Differences in wintering areas (M^{t}/M^{s})	Differences in wintering areas (M^{t}/M^{s})	0.733	0.285	20.64	47.37

With respect to mutation-drift equilibrium (d.f. = 22)	Single additional factor	M5 Differences in morphology (M) + (P)*	Differences in morphology (M) + (P)*	**0.005**	**0.020**	17.09	66.97
	Multiple additional factors	M5 (M) + (P) + h	(M) + (P)* + h	**0.001**	**0.041**	12.41	66.59

Variables in specific models: ***: $p < 0.001$, **: $p < 0.01$, *: $p < 0.05$; χ^2 : bold: $p < 0.05$; shaded values represent the best AIC within significant models for the overall group of models (e.g. multiple additional factors in comparison to single additional factor in M5).

M4a: M = Migratory species; M^{ϕ} = Partially-migratory species; R = Year resident species
M4b: M^{v}= Migratory species showing different wintering areas among colonies; R = Year resident species; M^{s}= Migratory species with overlapping wintering areas
M4c: M^{v}= Migratory species showing different wintering areas among colonies; M^{s}= Migratory species with overlapping wintering areas



SI. 6 – 13 Linear regression between genetic structure and the number of sample sites for 73 seabird species. A weak correlation was observed between F_{st} and the number of sample sites ($F = 0.873$, $p = 0.0354$, $R^2 = 0.015$).

SI. 6 – 14 Generalized linear models N1 – N4a–c of genetic structure in Northern Temperate species in relation to mutation-drift equilibrium where the AICs for all single predictors tested are shown. Results of GLMs for the models N1 – N4a–c in relation to mutation-drift equilibrium based on 26, 7, 15, 26, 6 and 6 species for which information on breeding phenology, morphology and non-breeding distributions were available respectively.

			Variables in specific model		χ^2		AIC	
Degree of freedom	Group of models	Model	F_a as binomial error distribution	F_a as normal error distribution	Binomial	Normal	Binomial	Normal
d.f. = 26	Single factor	N1	Land	Land	0.537	0.400	25.94	72.97
		N1	Sample sites	Sample sites	0.477	0.806	25.82	73.80
		N1	Genetic marker	Genetic marker	0.834	0.141	27.96	70.44
		N1	Family	Family	0.118	0.508	24.46	74.78
		N1	IUCN Status	IUCN Status	0.363	0.158	28.00	69.09
		N1	Population size	Population size	0.820	0.415	26.27	73.03
		N1	Sample size (n)	Sample size (n)	0.170	0.930	24.43	73.87
		N1	Distance	Distance	0.856	0.710	26.29	75.70
		N1	h	h**	0.183	**0.011**	24.21	61.68
		N1	π	π	0.081	0.562	22.93	70.99
	Single additional factors	N1	h + Sample sites	h*** + Sample sites	0.161	**0.021**	24.34	61.27
d.f. = 7	Single factor	N2	Differences in phenology	Differences in phenology	1	0.667	4	23.31
d.f. = 15	Single factor	N3	Differences in morphology	Differences in morphology	0.585	0.266	15.48	43.77
d.f. = 26	Single factor	N4a	Differences in movement patterns ($M/M^{\phi}/R$)	Differences in movement patterns ($M/M^{\phi}/R$)	0.052	0.133	22.55	70.87
d.f. = 6	Single factor	N4b	Differences in wintering areas ($(M^{y}+R)/M^{s}$)	Differences in wintering areas ($(M^{y}+R)/M^{s}$)	0.340	0.190	10.73	22.80
d.f. = 6	Single factor	N4c	Differences in wintering areas (M^{y}/M^{s})	Differences in wintering areas (M^{y}/M^{s})	0.340	0.190	10.73	22.80

Variables in specific model: ***: $p < 0.001$, **: $p < 0.01$, *: $p < 0.05$; χ^2 : bold: $p < 0.05$; shaded values represent the best AIC within significant models for the overall group of models (e.g., single factor in comparison to single additional factors in N1).

M4a: M = Migratory species; M^{ϕ} = Partially-migratory species; R = Year resident species

M4b: M^{y}= Migratory species showing different wintering areas among colonies; R = Year resident species; M^{s}= Migratory species with overlapping wintering areas

M4c: M^{y}= Migratory species showing different wintering areas among colonies; M^{s}= Migratory species with overlapping wintering areas

SI. 6 – 15 Generalized linear models T1 – T4a–c of genetic structure in Tropical species in relation to mutation-drift equilibrium where the AICs for all single predictors tested are shown. Results of GLMs for the models T1 – T3a–c in relation to mutation-drift equilibrium based on 9, 8, 7, 9, 6 and 6 species for which information on breeding phenology, morphology and non-breeding distributions were available respectively.

Group of models		Variables in specific model			χ^2	AIC	
Degree of freedom		Model	F_{st} as binomial error distribution	F_{st} as normal error distribution	Binomial	Normal	Binomial	Normal
d.f. = 9	Single factor	T1	Breeding sites separated by the Isthmus of Panama (IP)	Breeding sites separated by the Isthmus of Panama (IP)*	**0.029**	0.073	10.73	19.72
		T1	Sample sites	Sample sites	0.933	0.588	15.45	23.35
		T1	Genetic marker	Genetic marker	0.633	0.698	15.23	25.63
		T1	Family	Family	0.208	0.923	14.32	27.62
		T1	IUCN Status	IUCN Status	-	-	-	-
		T1	Population size	Population size	0.975	0.693	15.46	25.63
		T1	Sample size (n)	Sample size (n)	0.532	0.522	15.07	25.12
		T1	Distance	Distance	0.222	0.502	13.97	25.03
		T1	π	π	0.298	0.328	14.38	23.93
		T1	h	h	0.256	0.515	14.12	25.09
d.f. = 8	Single factor	T2	Differences in phenology (P)	Differences in phenology (P)	0.311	0.909	13.56	24.53
d.f. = 7	Single factor	T3	Differences in morphology (M)	Differences in morphology (M)*	**0.022**	**0.040**	7.82	17.17
d.f. = 9	Single factor	T4a	Differences in movement patterns (M/M$^{\phi}$/R)	Differences in movement patterns (M/M$^{\phi}$/R)	0.168	0.434	11.64	24.69
d.f. = 6	Single factor	T4b	Differences in wintering areas ((M^{s}+R)/M^{s})	Differences in wintering areas ((M^{s}+R)/M^{s})	-	-	-	-
d.f. = 6	Single factor	T4c	Differences in wintering areas (M^{s}/M^{s})	Differences in wintering areas (M^{s}/M^{s})	-	-	-	-

Variables in specific model: **: $p < 0.01$, *: $p < 0.05$; χ^2: bold: $p < 0.05$; shaded values represent the best AIC within significant models for the overall group of models.

M4a: M = Migratory species; M$^{\phi}$ = Partially-migratory species; R = Year resident species
M4b: M^{s}= Migratory species showing different wintering areas among colonies; R = Year resident species; M^{s}= Migratory species with overlapping wintering areas
M4c: M^{s}= Migratory species showing different wintering areas among colonies; M^{s}= Migratory species with overlapping wintering areas

SI. 6 – 16 Generalized linear models S1 – S4a–c of genetic structure in Southern Temperate species in relation to mutation-drift equilibrium where the AICs for all single predictors tested are shown. Results of GLMs for the models S1 – S4a–c in relation to mutation-drift equilibrium based on 22, 11, 14, 22, 11 and 8 species for which information on breeding phenology, morphology and non-breeding distributions were available respectively.

			Variables in specific model		χ^2		AIC	
Degree of freedom	Group of models	Model	F_{st} as binomial error distribution	F_{st} as normal error distribution	Binomial	Normal	Binomial	Normal
d.f. = 22	Single factor	S1	Presence/Absence (P/A) on 'refuge zones'*	Presence/Absence (P/A) in 'refuge zones'*	**0.017**	**0.044**	25.86	54.99
		S1	Sample sites separated by Antarctic/Subtropical Front	Sample sites separated by Antarctic/Subtropical Front	0.069	0.064	28.22	56.11
		S1	Sample sites*	Sample sites	**0.005**	0.150	23.66	58.36
		S1	Genetic marker	Genetic marker	0.249	0.308	32.88	59.11
		S1	Family	Family*	0.234	0.331	31.25	60.11
		S1	IUCN Status	IUCN Status*	0.188	0.097	30.74	54.37
		S1	Population size	Population size	0.730	0.890	31.40	61.37
		S1	Sample size (n)	Sample size (n)	0.056	0.893	27.87	61.37
		S1	Distance	Distance	0.219	0.634	30.02	61.10
		S1	π	π	0.187	0.591	28.99	59.49
		S1	h	h	0.899	0.974	30.72	59.87
	Single additional factors	S1	(P/A) in 'refuge zones' + Sample sites*	(P/A) in 'refuge zones'* + Sample sites	**0.002**	0.074	21.29	54.74
		S1	Sample sites* + IUCN*	Sample sites* + IUCN*	**0.005**	0.077	22.61	50.79
	Multiple additional factors	S1	Sample sites* + IUCN* + (P/A) on 'refuge zones'	Sample sites* + IUCN + (P/A) on 'refuge zones'*	**0.004**	**0.046**	19.37	44.75
		S1	Sample sites* + IUCN* + (P/A) on 'refuge zones'+ h	Sample sites + IUCN + (P/A) on 'refuge zones'**+ h	**0.001**	0.069	19.03	42.30
d.f. = 11	Single factor	S2	Differences in phenology (P)	Differences in phenology (P)	0.130	0.091	16.14	33.24
	Single additional factors	S2	Differences in phenology (P) + Family	Differences in phenology (P)** + Family**	**0.036**	**0.042**	14.73	24.32
d.f. = 14	Single factor	S3	Differences in morphology (M)	Differences in morphology (M)***	**0.006**	**0.016**	10.73	22.63
	Single additional factors	S3	Differences in morphology (M) + h	Differences in morphology (M)*** + h	**0.009**	**0.046**	10.12	22.00

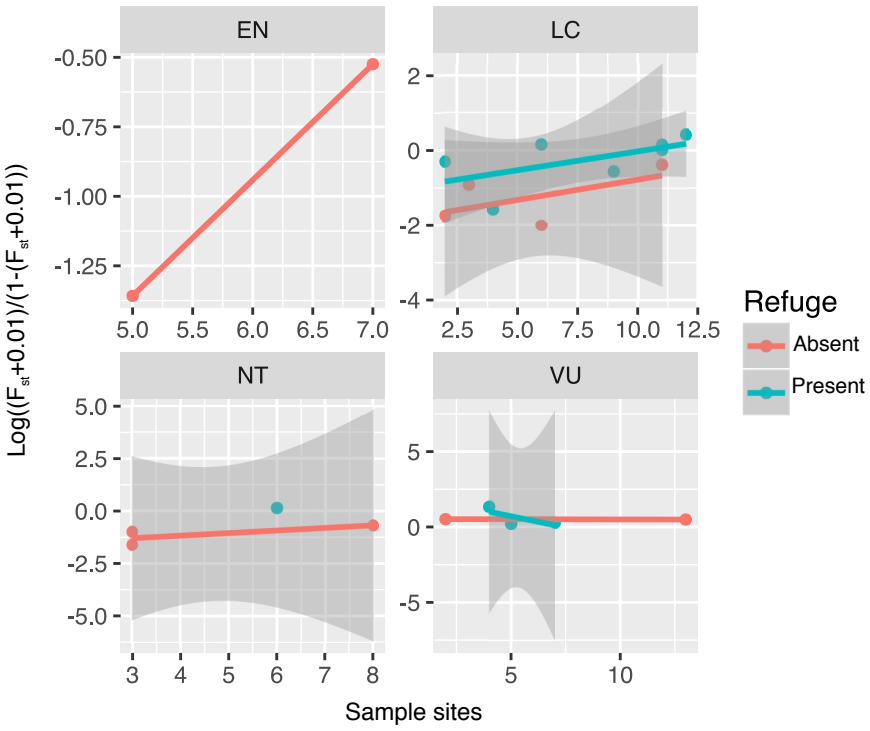
	Multiple additional factors	S3	Differences in morphology (M) + h + (P/A)	Differences in morphology (M)*** + h + (P/A)	**0.008**	0.055	10.09	24.15
d.f. = 22	Single factor	S4a	Differences in movement patterns ($M/M^{\phi}/R$)	Differences in movement patterns ($M/M^{\phi}/R$)	0.674	0.701	32.75	62.41
d.f. = 11	Single factor	S4b	Differences in wintering areas ($(M^{v}+R)/M^{x}$)	Differences in wintering areas ($(M^{v}+R)/M^{x}$)	0.385	0.136	16.14	33.04
d.f. = 8	Single factor	S4c	Differences in wintering areas (M^{v}/M^{x})	Differences in wintering areas (M^{v}/M^{x})	0.132	0.052	10.73	25.20

Variables in specific model: ***: $p < 0.001$, **: $p < 0.01$, *: $p < 0.05$; χ^2: bold: $p < 0.05$; shaded values represent the best AIC within significant models for the overall group of models (e.g., multiple additional factors in comparison to single and single additional factors in S1).

M4a: M = Migratory species; M^{ϕ} = Partially-migratory species; R = Year resident species

M4b: M^{v}= Migratory species showing different wintering areas among colonies; R = Year resident species; M^{x}= Migratory species with overlapping wintering areas

M4c: M^{v}= Migratory species showing different wintering areas among colonies; M^{x}= Migratory species with overlapping wintering areas



SI. 6 – 17 Multiple Linear regression showing the relationship among genetic structure (F_{st}), number of sample sites, IUCN status and presence of colonies in refuge zones for Southern Temperate species in relation to mutation drift equilibrium. This figure highlights the significant variations in IUCN status among Southern Temperate species. A = absence of sample colonies in refuge zones and P = presence of sample colonies in refuge zones.

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Chapter 7:

General discussion and concluding remarks

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7.1 Summary, synthesis and significance

A global overestimation of biotic processes as explanations of genetic differentiation among seabird colonies

This thesis had the aim of exploring whether biotic, physical or historical processes (e.g. historical fragmentations, bottlenecks) contribute to genetic differentiation among seabird populations by comparing 73 peer-reviewed studies reporting population genetic data for seabirds, including two comprehensive case studies of the genetic distinctiveness among colonies of two oceanic seabird species – the flesh-footed shearwater *Ardenna carneipes* and the providence petrel *Pterodroma solandri*. One of the major themes to emerge from my analyses is the global overestimation of biotic processes as explanations of genetic differentiation among seabird colonies.

In Chapter 2, I described HRM melting temperature range as new predictor of polymorphism that can be used to rapidly assess microsatellites polymorphism in any taxon, illustrated by assessing polymorphism at 27 microsatellite loci in providence petrel (*Pterodroma solandri*).

In Chapter 3, Isolation with Migration models implemented for one mitochondrial region and seven nuclear DNA fragments, indicated low gene flow and long divergence between flesh-footed shearwater *A. carneipes* colonies breeding east of Australia (Lord Howe Island and New Zealand) and western breeding colonies (Western Australia, South Australia, and Saint-Paul Island). The divergence between these colonies (~28,000 years) roughly corresponds to the beginning of the LGM. Despite suggestions from telemetry of distinct distributions of

individuals from western and eastern colonies during the non-breeding season, individuals sampled during the non-breeding period in the Sea of Japan were assigned to eastern and western colonies. As a result, genetic structure among flesh-footed shearwater colonies cannot be explained by distinct non-breeding distributions. Based on these observations, previous studies may have falsely invoked distinct non-breeding distributions for population genetic structure in seabirds in instances where there are limited data supporting distinction of non-breeding distributions.

In Chapter 4, I generated three genetic data sets consisting of DNA sequences from mitochondrial and 14 nuclear regions and genotypes from 10 microsatellite loci to investigate genetic connectivity of the two extant providence petrel colonies. High gene flow between the two remaining colonies of *P. solandri* (Lord Howe Island and Phillip Island) was evident despite individuals at the two colonies showing different time of return to nesting sites. Although analysis of mitochondrial DNA, nuclear introns and microsatellites do not specifically inform about the genetic basis of this behaviour, my results suggest high plasticity in behaviour rather than adaptive divergence in providence petrels.

In Chapter 5, a heterochronous sampling of modern and ancient *P. solandri* DNA showed that the majority of subfossil Norfolk Island individuals exhibited the most common mitochondrial haplotype from Lord Howe Island, consistent with high connectivity. This supports my previous findings suggesting that the nocturnal behaviour observed on Norfolk Island and Phillip Island might reflects phenotypic plasticity. This study also provides an insight into how rapidly even very large populations could be decimated despite genetic connectivity among populations, which has significant conservation implications for further studies attempting to predict the resilience of populations.

Understanding evolutionary processes and population dynamics within species is crucial to predict their long-term persistence and resilience to environmental perturbations (Avise and Hamrick 1996). This requires investigating factors inhibiting gene flow among populations to predict long-term viability of species (Chepko-Sade and Halpin, 1987; Ibrahim *et al*., 1996; Wright, 1931). However, signatures of climatic changes such as glacial successions (e.g. Last Glacial Maximum; LGM) and other historical fragmentations (e.g. Isthmus of Panama) that disrupt gene flow often dominate in the quantification of among-population genetic differentiation, introducing a potential bias in the assessment of contemporary predictors of population genetic structure – e.g. anthropogenic fragmentation of the habitat (Goossens *et al*., 2006) or behavioural variation among colonies (Smith and Friesen, 2007) – (Wright, 1931). Hence, comparison of multiple species that have experienced similar historical conditions is desirable to test hypotheses regarding factors other than historical barriers, such as ecological and life-history characteristics that might also influence genetic connectivity among populations. In Chapter 6, I showed that for a high proportion of seabirds, mtDNA variation is not at mutation-drift equilibrium, indicating likely historical influences on population genetic differentiation. Furthermore, when restricting my analyses to datasets in equilibrium, historical and physical factors still appeared the best predictors of genetic structuring. Although hierarchical comparisons of genetic partitioning showed that all Southern Temperate species sampled on the Falkland Islands exhibited the highest change in allele frequency around that zone, these results show that signatures of historical events still dominate as contributors to genetic structuring among seabird colonies. Therefore, it is recommended that mtDNA studies of the biotic factors influencing gene flow among seabird populations should also be assessed in conjunction with an extensive knowledge of potentially influential historical processes to avoid an overestimation of the impact of

contemporary processes when identifying conservation priorities to maintain viability of species (Avise and Hamrick, 1996).

Quantifying the impact of mutation-drift equilibrium on genetic structure among populations

Perturbed populations are often the focus of conservation concerns but they pose challenges for population genetics because mutation-drift equilibrium is unlikely (Amos and Balmford, 2001). Gene flow is often estimated using Wright's (1943) F_{st} approximation despite the acknowledgement that the method is potentially biased under nonequilibrium conditions and other violations of an infinite island model (Whitlock and McCauley, 1999). Indeed, if populations have not yet reached equilibrium, this method will generally overestimate the current amount of gene flow (Chikhi *et al*., 2010). For example, from a genetic point of view, recovery time of populations that have experienced bottleneck events to the previous level of genetic diversity is relatively slow as opposed to the demographical recovery time (Avise and Hamrick, 1996). Therefore, empirical data in these populations will show low genetic variability even if they have recovered to their pre-bottleneck population size (Avise and Hamrick, 1996). Furthermore, the evolutionary dynamics of molecular markers and adaptive traits are likely to be different simply because the rate at which new variants are introduced are also quite different (Avise and Hamrick, 1996; Pertoldi and Topping, 2004). Consequently, it is recommended to incorporate neutrality simulation approaches – e.g. Tajima's D neutrality tests (Tajima, 1989) – in multi-species genetic comparison as a mean of addressing non-equilibrium conditions posed by historically perturbed populations (Kinnison *et al*., 2002).

Here, I showed that deviation from mutation drift equilibrium is widespread in seabird species (19%), with significantly lower F_{st} observed, underestimating the overall quantification of genetic structure. Locations of species lacking of mutation-drift equilibrium are consistent with regions hypothesized as Pleistocene refugia in other Arctic vertebrates such as the southern edge of the Bering Land Bridge, Newfoundland Bank and Spitsbergen Bank (Avise, 2000), which were supported by the characterization of higher genetic diversity in several seabird colonies sampled in those regions (e.g. Moum and Arnason, 2001; Sonsthagen *et al*., 2012).

Although Tajima's D neutrality test can be affected by several factors such as substitutional rate heterogeneity and population subdivision, the significance sign of the statistic is a relatively robust predictor of demographic changes such as population expansions or bottlenecks (Ramírez-Soriano *et al*., 2008). However, I noted that several additional species showed a negative Tajima's D statistic even if they were not significant, which means that the influence of this predictor on F_{st} in our multi-species analyses may be underestimated. These findings reinforce my hypothesis suggesting that lack of mutation-drift equilibrium due to historical processes in seabird species introduces a bias in the quantification of genetic structure among seabird populations.

Segregation in non-breeding distribution not identified as an inhibitor of gene flow among seabird colonies

It has been suggested that seabird colonies that migrate to population-specific non-breeding areas may have less opportunity for gene flow than those that have a single common non-breeding area or simply disperse widely during the non-breeding season (Friesen *et al*., 2007; Friesen, 2015). Here, my multi-species comparison based on information on non-breeding

areas for 20 species failed to show such relationship. Moreover, 88% of species with distinct non-breeding areas exhibiting significant genetic structuring have likely experienced historical fragmentation, suggesting a confounding effect of differences in non-breeding distributions and genetic structure, the latter rather reflecting historical processes.

The confounding effect of differences in non-breeding distributions and genetic structure among seabird colonies may be due to two major factors. First, detailed observations of foraging movements are provided by telemetry studies, but these are typically restricted to a low number of individuals over a relatively short time interval (e.g. a single season), producing temporally and spatially limited insights at best (Genovart *et al*., 2007). Small rates of gene flow can strongly influence population genetic structure (Mills and Allendorf 1996; Slatkin 1987), and therefore foraging observations from a small number of individuals may be uninformative about rarer individual movements. For example, despite suggestions from telemetry of distinct routes of migration and distribution of individuals from western and eastern colonies of flesh-footed shearwater during the non-breeding season, individuals sampled during the non-breeding period in the Sea of Japan were assigned to eastern and western colonies. As a result, the presence of genetic structure among flesh-footed shearwater colonies can hardly be explained by distinct non-breeding distributions.

The second factor is an underestimation of the importance of a detailed spatial sampling in the assessment of genetic structure among populations (Hellberg *et al*., 2002). Potential contemporary inhibitors of gene flow may only rely on a subset of populations. Hence, in the context of multi-species analyses, quantifying genetic structure among populations over the entire sampling range of species may introduce a bias in the assessment of contemporary predictors of gene flow. For example, in the case of the white-chinned petrel *Procellaria*

aequinoctialis, individuals breeding in Crozet Island have been observed to winter on the coast of South Africa and individuals breeding in South Georgia use the northern Patagonian Shelf during the non-breeding season (Weimerskirch *et al*., 1999), yet genetic homogeneity exists among them, with genetic heterogeneity elsewhere in the species range corresponding to the presence of sampled colonies on Campbell Island identified as a refuge zone. Therefore historical factors may better explain seabird genetic structuring among *P. aequinoctialis* colonies. Another example is the case of the wandering albatross *Diomedea exulans*. The global F_{st} observed among seven *D. exulans* colonies was high (F_{st} = 0.639; Burg and Croxall, 2004). Strong evidence of variation in non-breeding distribution was observed among two colonies (Crozet and Kerguelen Islands; *Weimerskirch et al*., 2015). However, these two colonies are genetically undifferentiated such that the high global F_{st} observed for this species can hardly be explained by the distinct non-breeding distributions between these colonies. Consequently, it is recommended to avoid using the global F_{st} observed in a species only to assess predictors of gene flow among colonies in the context of multi-species comparisons.

Although differences in non-breeding distributions are not identified *per se* as inhibitors of gene flow among seabird colonies, it must be noted that the highest genetic partitioning of all species that were sampled from the Falkland Islands (unglaciated during the Pleistocene) were identified around that zone, including species whose sampling range included northern islands (Gough Island and Tristan da Cunha Island). This is surprising given that the disjunction of sampling range by the Antarctic Frontal Zone, a relatively mobile climatic boundary (Hall, 1990), has been proposed as an isolating mechanism of seabird colonies during the last 0.9 Ma (e.g. Burg and Croxall, 2004; de Dinechin *et al*., 2009). Hence, one would have expected the highest genetic partitioning (e.g. deeper genetic divergence) among colonies to occur around that zone. On the other hand, as the Antarctic Frontal Zone has

moved a lot during the last 20 ky, one might aslo expect islands close to the front to experience intermittent connectivity and therefore lower levels of divergence. Although this observation suggests that proximity to key foraging areas such as the Patagonian Shelf could be considered another important contributor to the maintenance of seabird colonies through time, and hence development of genetic divergence, the presence of ice-free refuge zones appears more important. Indeed, of the five species that were sampled on South Georgia (glaciated during the Pleistocene) but not on the Falkland Islands, only one showed significant F_{st}. This confirms a strong impact of historical processes on the genetic structuring of seabird species regardless of proximity to fertile foraging zones.

Phenotypical traits are likely the 'consequences' of historical fragmentation rather than the 'causes' of genetic structure among seabird colonies

Comparison of multiple seabird species that have experienced similar historical conditions shows that historical fragmentation is the best predictor of genetic differentiation within Tropical and Southern Temperate species, and is supported by variation in phenotypic traits. These findings highlight another misinterpretation: a false correlation between genetic structure and phenotypical variation relying, in fact, on previous historical processes. Indeed, phenotypical adaptations appear to be more likely the 'consequences' of historical fragmentations rather than the 'causes' of genetic structure among populations. Phenotypic variation likely 'co-evolved' with the genetic differentiation observed among seabird colonies, which was promoted by historical fragmentation, but did not act as the primary inhibitor of gene flow among populations. This hypothesis is reinforced by several examples where genetic structuring was lacking among colonies exhibiting phenotypic variation. For example, masked boobies *Sula dactylatra* show morphological variation among eastern

Pacific colonies (Pitman and Joseph, 1998), yet are genetically homogeneous, with genetic heterogeneity observed elsewhere in the species range corresponding to the presence of the Isthmus of Panama (Steeves *et al*., 2003). Hence, misinterpretation of the importance of phenotypic variation may be also due to an underestimation of the importance of a detailed spatial sampling in the assessment of genetic structure among populations (Hellberg *et al*., 2002), as previously discussed.

Another example is my first case study on the providence petrels. Despite providence petrel colonies being highly connected genetically, Lord Howe Island individuals predominantly arrive at the colony during daylight, whereas Phillip Island individuals return to their breeding sites only after dusk for the period of courtship and early incubation. However, petrels showing diurnal arrival are also able to use olfaction as the basic sensory input for homing at night, and use it if necessary (Dell'Ariccia and Bonadonna 2013), which implies that all petrels are able to return to their burrows at night, and that individuals alter their behaviour to environmental conditions without necessarily requiring genetic adaptation.

One potential confounding factor between genetic structure and phenotypical traits is the occurrence of morphological differences not corroborated by evidence of reciprocal monophyly for DNA alleles. For example, my observations of spatial genetic variation between eastern and western flesh-footed shearwater colonies are consistent with the observation of previous morphological differences. However, flesh-footed shearwaters from eastern and western colonies do not show reciprocal monophyly for mtDNA alleles, which could be explained by the rapid evolution of phenotypic variation compared to sorting of MtDNA variation in abundant taxa, which can take tens of thousands of years (Avise 2000). As a result, phenotypic differences may be falsely invoked as the main inhibitor of gene flow

among flesh-footed shearwater colonies. However, variation in sea levels in Bass Strait following the LGM may have affected both phenotypic variation and the genetic structure observed among colonies.

Although these observations discriminate phenotypic adaptations as the 'primary' cause of reduced gene flow among seabird populations, these results must be taken with cautions. Indeed, rather than suggesting that adaptations to environmental conditions do not inhibit connectivity among seabird colonies, these findings advocate that the signature of historical fragmentation dominates that of phenotypical variation in the quantification of genetic structure among colonies given that they reflect much longer timescales. Indeed, 50% of Northern Temperate species whose breeding range was not fragmented by land and exhibited a significant F_{st}, variation in morphology and breeding phenology, were sampled or were observed to forage along a latitudinal gradient of ocean productivity, which may reflect adaptations to pronounced heterogeneity in foraging environments (Wolf *et al*., 2009). Therefore, the localization of land causing latitudinal gradients in dispersal (e.g. morphological specialization for foraging or the influence of latitude on phenology), in addition to the presence of land itself, could explain genetic structure among colonies, as has been observed in other taxa (Kelly and Eernisse, 2007; Salisbury *et al*., 2012). However, as I did not test 'latitudinal distribution' as a potential predictor of genetic structure in the present thesis, this relationship should be further re-evaluated.

Genetic connectivity can fail to predict long-term viability of species

The finding that high connectivity can buffer genetic diversity in the face of a demographic decline has been reported in several taxa (Jangjoo *et al*., 2016). However, this concept can have very different meanings and implications depending on how it is measured (Lowe and

Allendorf, 2010), and genetic methods alone provide little information on demographic connectivity defined as the degree to which population growth is affected by dispersal (Lowe and Allendorf, 2010). The capacity of connectivity to prevent population extinction will depend on several factors including social, behavioural and demographic characteristics that affect the likelihood of survival and reproduction of immigrants (Kleiman, 1989; Short *et al.*, 1992). Indeed, greater numbers of randomly chosen immigrants may be necessary to achieve the same genetic effect as fewer migrants chosen to maximize contribution to effective population size (Kleiman, 1989; Mills and Allendorf, 1996). Moreover, the origin of immigrants, how many individuals immigrate, and whether the migrants settle in unoccupied or occupied patches may also affect the benefits of immigration (McCauley, 1991; Lowe and Allendorf, 2010).

Although the potential for dispersal will likely help the persistence of populations in the short-term, long-term viability is dependent on the life-history traits of a species. In the case of *P. solandri*, the selection applied to the Norfolk Island population was strong. The population was estimated at ~1,000,000 breeding pairs before March 1790 when the HMS *Sirius* was wrecked on a reef at Norfolk Island. During the next four months, the shipwreck survivors avoided starvation by slaughtering hundreds of thousands of providence petrels. Harvesting by humans for food was estimated to have been conducted at a rate of 1600 per night during the four month breeding season, during years 1790 – 1793 (Medway, 2002). Harvesting was senseless and improvident, not only destroying adults, but also juveniles, eggs and burrows as well. Hence, it is not surprising that the genetic connectivity among Providence petrel colonies failed to prevent the extinction of the Norfolk Island colony under those conditions.

The case study on providence petrels shows a lack of correlation between genetic connectivity and population extinction. This suggests that genetic indices of connectivity are valuable for assessing the evolutionary consequences of dispersal, and hence the risks of population extinction, as long as they are accompanied with information on demographic connectivity among populations promoting metapopulation stability (Lowe and Allendorf, 2010). Hence, despite the amazing advances in the accessibility and resolution of population genetic data in the last decades, it is recommended that information on genetic connectivity is balanced by a clear understanding of the limitations of the data and openness to ways of addressing those limitations.

7.2 Recommendation for further research

In this thesis, I showed that a significant proportion of seabird's mtDNA variation is not at mutation-drift equilibrium, indicating likely historical influences on population genetic differentiation. Furthermore, when restricting my analyses to datasets in equilibrium, historical factors still appeared the best predictors of genetic structuring. Therefore, it is recommended that mtDNA studies of the contemporary factors influencing gene flow among populations is assessed in conjunction with an extensive knowledge of potentially influential historical processes to avoid an overestimation of the impact of contemporary processes when identifying conservation priorities to maintain viability of species (Avise and Hamrick, 1996). Other methods designed to detect contemporary gene flow – e.g. IM (Hey and Nielsen, 2004) – can help identify the signature of contemporary gene flow among populations. However, these approaches come with their own sets of assumptions and circumstances under which they are not applicable and might be hard to perform for a large set of species in the context of multi-species comparisons.

One specific recommendation for future multi-species genetic surveys is to avoid using only global indices of genetic structure (e.g. F_{st}) in the assessment of the evolutionary dynamics of species. Indeed, different events of divergence often occurred at different timescales along the sampling range of a species (e.g. de Dinechin *et al*., 2009). Hence, including pairwise F_{st} in multi-species comparison can help detect shared events of divergence among species that have experienced similar historical conditions. Moreover, ecological characteristics that may represent potential predictors of gene flow may only relate to a subset of the entire sampling range of the studied species. Hence, using global F_{st} rather than hierarchical genetic partitioning among populations may induce Type I and II errors when testing correlations between predictors of gene flow and genetic structure among populations. As a result, it is relevant to include more detailed spatial sampling in multi-species analyses, which can help investigate the accurate predictors of gene flow among populations.

Although many research questions can be answered with data on genetic connectivity, in this thesis I showed that genetic connectivity is not always a predictor of long-term viability of species. Consequently, it is recommended that genetic methods is combined with data on the origin and importance of the threat to the populations, population growth rates, occupancy, movement behaviour or individual reproductive success, when available, to address conservation issues such as the long-term viability of populations and species.

Finally, as mtDNA can be under strong selection and evolve under unusual evolutionary rules (Ballard and Whitlock, 2004), similar analyses should be conducted on nuclear datasets, although presently these greatly lag behind mtDNA in their abundance.

7.3 References

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